Genetics and Epilepsy Surgery:
The Road to Cure



M.W.C.B. Sanders

Genetics and Epilepsy Surgery:
The Road to Cure

Genetica en epilepsiechirurgie: op weg naar genezing
(met een samenvatting in het Nederlands)

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Maurits Willem Christiaan Benjamin Sanders

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Promotor:
Prof. dr. K.P.J. Braun

Copromotoren:
Dr. F.E. Jansen
Dr. B.P.C. Koeleman

Beoordelingscommissie:
Prof. dr. E.J. van Dijk
Prof. dr. H.J.M. Majoie
Prof. dr. J.P. van Tintelen (voorzitter)
Prof. dr. J.H. Veldink
Prof. dr. G.J.M. Zijlmans

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1

General introduction

In 1873, John Hughlings Jackson wrote on epilepsy that '*the convulsion is but a symptom; the disease is the condition of the nervous system which makes it liable to such discharges*'.[1] His formulation shifted the focus from the observable seizure to its underlying cause. A century and a half later, this principle remains central to the presurgical evaluation of focal epilepsy: how do we identify and delineate the substrate that gives rise to seizures?

Epilepsy surgery is the only potentially curative treatment for patients with drug-resistant epilepsy.[2-4] Surgical candidacy has traditionally been guided by clinical semiology, EEG, and neuroimaging. Identifying a structural lesion (preferably visible on MRI, concordant with electroclinical findings, and located outside eloquent areas) has long been the key criterion for proceeding to surgery.[3,5] With the exception of palliative procedures, epilepsy surgery aims to completely remove or disconnect the epileptogenic zone.[3]

Until recently, genetic diagnostics played no formal role in the presurgical evaluation of patients, particularly children, with drug-resistant focal epilepsy. When performed, genetic testing was generally limited to patients with epileptic encephalopathies, suspected syndromic disorders, or those with several affected family members.[6-8] In the context of focal epilepsy without overt syndromic features, genetic testing was largely considered irrelevant.

This perspective has shifted with the advent of next-generation sequencing and an expanding understanding of the genetic architecture of epilepsy.[9] Both germline and somatic variants have been identified across the epilepsy spectrum, including in patients with focal epilepsy.[10] These variants may occur in patients with MRI-visible lesions as well as those who are MRI-negative, and even in cases where the resected tissue appears histopathologically unremarkable.[11-13] Such findings challenge the traditional dichotomy between 'structural' and 'genetic' aetiologies, and demonstrate that these categories often overlap.[11-17]

Despite these advances, the role of genetic diagnostics in surgical decision-making remains incompletely defined. There is a growing need to know in which cases genetic testing is clinically useful, how its results should be weighed against other presurgical data, and what impact it has on timing of surgery, patient selection, and surgical outcome.[18,19] A genetic diagnosis may clarify the aetiology of a lesion, but can it help predict seizure-freedom following surgery, or identify patients unlikely to benefit from resection? Should genetic testing be implemented early in the work-up? Can a genetic diagnosis justify continuation or early cessation of invasive monitoring?

The central question of this thesis thus arises: how and when should genetic testing be incorporated into the presurgical work-up of patients with drug-resistant focal epilepsy? Although early surgical intervention is known to improve outcome,[4,19,20] significant delays still occur, partly due to diagnostic uncertainty. Improved integration of genetic data could optimise patient selection, timing, and outcome.

Genetic diagnostics in the presurgical evaluation

This thesis addresses the clinical relevance of genetic diagnostics in epilepsy surgery across three interrelated domains. The first part examines the role of genetic testing in patients (mainly children) undergoing presurgical evaluation for focal epilepsy. **Chapter 2** reviews the currently known genetic architecture of epilepsy, highlighting mechanisms potentially relevant for surgical candidacy and outcome. **Chapter 3** presents a systematic review of existing literature on epilepsy surgery in patients with an established genetic diagnosis, while **Chapter 4** reports on a single-centre cohort of patients evaluated for epilepsy surgery; the study explores how genetic testing was applied, what it yielded, and how it influenced clinical decision-making.

Genetic mechanisms underlying focal epilepsy

The second part of the thesis investigates genetic mechanisms in focal epilepsy. **Chapter 5** focuses on GATOR1-related focal epilepsy, describing familial cases with divergent clinical phenotypes. **Chapter 6** provides an analysis of pooled deep sequencing data from patients with suspected malformations of cortical development (MCD); it characterises both the somatic variant landscape across different histopathological subtypes (including focal cortical dysplasia [FCD]) and examines how these variants relate to clinical, radiological, and histopathological features.

Timing, outcome and clinical integration of genetics

The third part addresses determinants of both surgical outcomes and the timing of surgery. Early intervention is associated with improved outcomes. Yet, significant delays still occur in the presurgical phase of many patients. **Chapter 7** establishes determinants of the postoperative seizure outcomes in patients with presumed non-lesional focal epilepsy (defined by normal MRI and absence of histopathological abnormalities) who had undergone epilepsy surgery. **Chapter 8** describes how factors such as drug resistance, imaging findings, and histopathology relate to the time interval between epilepsy onset, referral, and resective surgery.

Across all parts, this thesis aims to define the clinical relevance of genetic diagnostics in epilepsy surgery, clarifying not only how genetic information is interpreted, but also when it influences clinical decision-making, alters timing of surgery, or may change therapeutic decisions. By integrating genetic data with imaging, pathology, and outcome measures, this thesis seeks to improve the determination of surgical candidacy in an evolving genetic era.

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Part I

2

Genetics in epilepsy surgery

Maurits W.C.B. Sanders, Floor E. Jansen
Bobby P.C. Koeleman, Kees P.J. Braun

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ABSTRACT

The role of genetics in the etiology of generalized as well as focal epilepsies has become increasingly acknowledged in recent decades. Linkage studies on familial epilepsies provided the basis for human epilepsy gene discovery. In 1995, Sanger sequencing (first described in 1977 and for many years the most widely used sequencing method) enabled the identification of *CHRNA4* as the first pathogenic gene variant associated with epilepsy, specifically autosomal dominant nocturnal frontal lobe epilepsy, more recently renamed as sleep-related hypermotor epilepsy (SHE).[1] Many disease-causing variants in other genes, initially those related to channel function, have been identified since. Examples of these, in chronological order of discovery, include *SCN1B, KCNQ2, KCNQ3, SLC2A1, SCN1A, GABRA1*, and *GABRG2*.[2-8] These discoveries would eventually form the genetic basis for human, previously called idiopathic epilepsy.

Since the early 2000s, microarray techniques have been widely used to detect large (> 1 kb) deletions and duplications, so-called copy number variants (CNVs), of small parts of chromosomes that can include single or multiple genes. The introduction of array comparative genomic hybridization and single nucleotide polymorphism microarrays led to efficient, systematic discovery of CNVs in patients with epilepsy. Studies have shown that pathogenic CNVs can be identified in over 5% of patients with different major classes of epilepsy, such as populations with generalized epilepsy, focal epilepsy, or epileptic encephalopathy.[9-11] Furthermore, some CNVs that are not primarily considered to be the cause of the epilepsy syndrome are more commonly found in patients with epilepsy compared to controls and are therefore likely to act as risk factors for developing epilepsy. These CNVs are referred to as susceptibility CNVs. Well-known examples of these are deletions on chromosomes 15q11.2, 15q13.3, and 16p13.11 and deletions and duplications on chromosome 1q21.1 and 16p11.2.[12-14]

The introduction of NGS in 2008 represented an entirely new principle of sequencing technology following Sanger (first-generation) sequencing. It became possible to sequence multiple genes, the whole exome, or the whole genome in a single analysis. This enabled the identification of many novel candidate genes for

epilepsy on an exome-wide scale. The introduction of NGS accelerated the discovery of novel epilepsy genes in different cohorts of patients often selected based on their phenotype.[15-16] In particular whole exome sequencing (WES), through which the coding sequence of all genes in the human genome can be scanned for apparent disease-causing sequence variations, accelerated discovery of novel epilepsy genes in the past decade. The introduction of WES solved the genetic basis of many sporadic epilepsies in severely affected individuals, in particular the so-called epileptic encephalopathies. In these individually rare epilepsies, a genetic basis was long expected, but remained elusive due to the difficulty of gene discovery. Through WES, it was shown that a major proportion of these patients carried a de novo mutation, a new mutation occurring in a germ cell of one of the parents that proved to be causative for epileptic encephalopathy. This new paradigm explained many syndromic forms of epilepsy, and today, more than 500 genes are known to be causally related to epilepsy.[17] These comprise genes that cause so-called 'epilepsy-only' phenotypes, and genes that underlie syndromes with epilepsy as the core symptom or just as one of many manifestations.

Furthermore, metabolic disorders and malformations of cortical development (MCDs), both well-known etiological categories of epilepsies, may be caused by single gene mutations.[17] Within the group of focal epilepsies, the most important novel gene discoveries have been made in the mTOR pathway. Mutations in this pathway cause focal epilepsies due to MCDs, for example, TSC as well as non-lesional focal epilepsy subtypes.[16,18] In addition, WES allowed the identification of novel somatic mutations; these are post-zygotic mutations detected in the abnormal (resected) tissue of 30 to 50% of MCD cases.[16,18,19]

Neurobiological pathways in genetic epilepsy

Gene products in epilepsy are part of different pathways. The first human epilepsy gene discovery led to the concept of different channelopathies as pathophysiological mechanisms for the development of epilepsy.[15,20] In 2002, however, *LGI1* was discovered as a non-ion-channel-coding causative gene for autosomal dominant partial epilepsy with auditory features (ADPEAF), a rare form of lateral temporal lobe epilepsy. Since then, many non-ion-channel-coding genes for different epilepsy phenotypes have been identified, implicating a broad range of disease mechanisms. Important pathways, apart from those that involve ion channels, include synaptic transmission dysfunction, transporter defects, transcriptional dysregulation, impaired DNA repair, axon myelination, interneuron migration, interneurite formation, and cell–cell adhesion and metabolic defects (**table 1**).[21]

Gene products within the same pathway can present with an indistinguishable phenotype. For example, *DEPDC5* was identified as a causal gene in the mTOR pathway causing focal epilepsy associated with cortical brain malformations.[22] Sequencing of other genes in this pathway led to the identification of pathogenic variants in *NPRL2* and *NPRL3*, which together with *DEPDC5* are the components of the GATOR1 complex.[23] Involvement of any of the genes of this complex is associated with similar types of epilepsy. To date, mutations in the GATOR1 complex genes are thought to explain at least 11% of focal epilepsies.[18]

On the other hand, a remarkable and well-described phenomenon associated with almost all epilepsy genes is their variable clinical expression, or phenotypic heterogeneity. This variability in phenotype can be subtle, but can also include the two extremes of disease severity. For example, pathogenic variants in the gene encoding the voltage-gated sodium channel (*SCN1A*) are related to epilepsy syndromes characterized by fever sensitive seizures, ranging from relatively mild phenotypes apparent in families with genetic epilepsy with febrile seizures 'plus' (GEFS+), to severe epileptic encephalopathy (Dravet syndrome, or severe myoclonic epilepsy of infancy; SMEI).[24] An extensive body of research on this subject has been published and to date more than 600 sequence variants of the *SCN1A* gene have been identified, enabling detailed genotype-phenotype correlations that explain the *SCN1A* phenotypic heterogeneity by focusing on the mutation type and location.[25-26] Other genes with reported mutations related to this phenotype are *SCN1B* and *GABRG2*.[2]

Self-limiting epilepsies in newborns and infants represent a group of relatively rare syndromes that are transmitted with an autosomal-dominant inheritance pattern and are characterized by clusters of focal seizures occurring in the first year of life and eventually disappearing spontaneously. These epilepsies have been associated with pathogenic variants in the *KCNQ2* and *KCNQ3* gene (encoding the voltage-dependent K^+ channel) related to the clinical syndrome of benign familial neonatal seizures.[27] However, de novo mutations in specific domains of *KCNQ2* can lead to a very severe epileptic encephalopathy, with onset in the neonatal period. Phenotypic heterogeneity has even been reported within single families. For example, family members that carry the same pathogenic variants in *KCNT1* may present with either autosomal-dominant sleep-related hypermotor epilepsy (ADSHE) or the severe and early onset epilepsy of infancy with migrating focal seizures.

Variability of the severity of the disease is not always completely explained by type and location of the mutation, which led to speculation of disease modifiers. This is especially true for Dravet syndrome caused by *SCN1A* mutations. Several GEFS+ families have been described in which a single family member who suffers from Dravet syndrome carries the same mutation as family members with the more benign GEFS+ phenotype.[28] Also, within sporadic Dravet patients a range of disease severity can be observed that is unexplained by type and location of the mutation. The hypothesis of a second hit in a modifier gene is supported by several studies implicating a range of genes. Finally, a recent study presented evidence that ~8% of Dravet patients are mosaic for the

Table 1. Major epilepsy-associated genes classified according to assumed neurobiological pathway (based on McTague, et al. 2016).[21]

epilepsy gene	neurobiological pathway	
SLC2A1	glucose transporter	**blood-brain barrier**
SLC35A2; ST3GAL3; SLC12A5; SLC13A5; SLC25A22; QARS, AARS; PIGA; PIGQ; PIGO;	protein translation and modification	**neuron**
CDKL5; FOXG1; ARX; MEF2C; PNKP; CHD2; SIK1; MECP2; LGI1	transcription, DNA repair and modification, synaptogenesis	
PIK3CA; PIK3R2; AKT3; MTOR; DEPDC5; NPRL2; NPRL3; PTEN; TSC1; TSC2	PI3K/AKT/mTOR pathway: cell growth, synaptic plasticity, etc.	
ARX; LIS1; DCX; TUBA1A; TUBB2B; GPR56; FLNA; NEDD4L; RELN, CNTNAP2	neuronal migration, neurogenesis	
PCDH19	cell-cell adhesion	
WWOX; WDR45	apoptosis, autophagy	
ALG1	glycosylation	
CHD2; IQSEC2; PNPO; PRICKLE1; HDAC4	other	
SPTAN1	axon myelination	**axon**
SCN1A; SCN2A; SCN8A; SCN1B; SCN9A	Na^+ channel	
KCNQ2; KCNQ3	K^+ channel	
CACNA1A; CACNA2D2	Ca^{2+} channel	
DNM1; NECAP1; TBC1D24;	exo-endocytic cycling of vesicles	**presynaptic membrane**
STXBP1; GOSR2; PRRT2	SNARE complex	
KCNT1	K^+/Na^+ channel	
KCNA2; KCNB1	K^+ channel	
GABRG2; GABRA1; GABRB3; GABRD	GABAA receptor	**postsynaptic membrane**
GRIN1; GRIN2A; GRIN2B	NMDA receptor	
CHRNA4; CHRNA2; CHRNB2; PRIMA1	nACh receptor	
DOCK7; SYNGAP1; ARHGEF9; GNAO1; PLCB1	g-protein-coupled transduction	
IP3; DAG: PKC	second messengers	
HCN1	K+/Na+ (HCN) channel	

SCN1A mutation and therefore have milder phenotypes.[28]
For several known pathogenic variants, genotype–phenotype correlations remain unclear.[29] In general, the mechanisms underlying phenotypic heterogeneity may be related to somatic mosaicism, variant type, other genetic/epigenetic factors, or differential expression of alternative gene transcripts.

Genetic principles in nonstructural and structural epilepsies

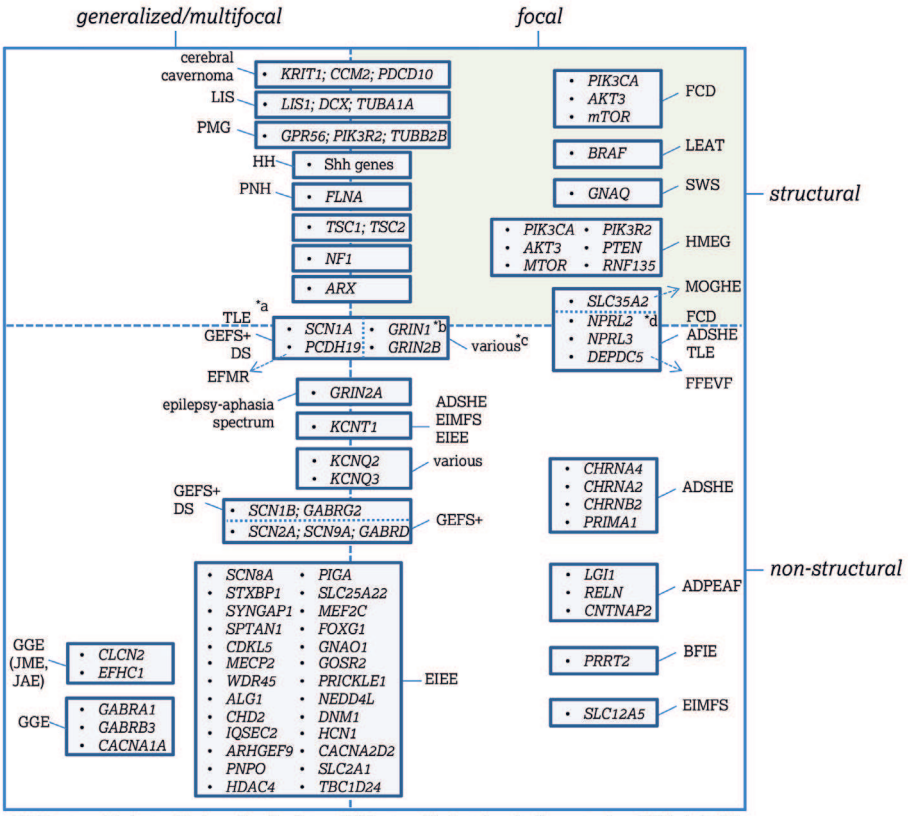
Epilepsy has been historically divided into two main categories, referring to its etiology: (1) (presumed) symptomatic and (2) 'idiopathic' epilepsy. Symptomatic epilepsies are caused by an obvious structural brain abnormality, including both MCDs and acquired brain lesions, or by a metabolic, inflammatory, or degenerative disorder. Epilepsy was classified as 'presumed symptomatic' (or 'cryptogenic') when a brain abnormality was suspected but could not be detected by available diagnostic investigations. Symptomatic epilepsies can either have a genetic basis, or can be determined by external factors, or have a combined origin. The remainder of the patients were in the past classified as 'idiopathic epilepsies' when there was no obvious known underlying cause, other than a presumed genetic predisposition. Over the past decades, studying genetics in epilepsy in both categories has proved essential for clinical purposes as well as for understanding of the underlying mechanisms.[30] Nowadays, the new International League Against Epilepsy classification requires a thorough diagnostic work-up in all epilepsy patients for the following etiological categories: (1) structural, (2) genetic, (3) metabolic, (4) infectious, (5) immune, and (6) unknown cause, and the previous classification of epilepsies into symptomatic or idiopathic subtypes has been abandoned.[31]

Nonstructural epilepsies

The genetic basis of numerous nonstructural epilepsy syndromes has become progressively unraveled, varying from confirmed monogenic causes (single gene defects) with a Mendelian inheritance pattern to complex epilepsy with presumed polygenic or complex inheritance that contributes to disease susceptibility. Clinically, nonstructural epilepsy syndromes encompass a wide phenotypic spectrum of typically generalized or multifocal semiology, but can also include focal seizure types, often with a phenotypic overlap between different associated genes (**figure 1**).

An important broad clinical spectrum of nonstructural epilepsies includes the early onset epileptic encephalopathies, with manifestations varying from (clusters of) focal or migrating focal seizures to epileptic spasms and tonic or tonic-clonic seizures, typically in combination with a severe and early onset developmental delay. In many of these epileptic encephalopathies, the epileptic activity itself is thought to contribute to the severity of the cognitive problems. These epileptic encephalopathies can be caused by pathogenic variants in ion channel genes such as *SCN1A*, *KCNQ2*, and *KCNT1*, as well as in non-ion-channel-coding genes such as, *ARX*, *CDKL5*, *SLC25A22*, and *SPTAN1*.[32–34] On the other hand, pathogenic variants in genes have been identified in patients with epilepsy and encephalopathy in whom the severe encephalopathy continued after seizure control. Examples of these genes are *STXBP1*, *MECP2*, and *GRIN2A*.[35]
The idiopathic (or today called genetic) generalized epilepsies (IGEs or GGEs) comprise a large group of epilepsies and consist of several syndromes including childhood absence epilepsy, juvenile absence epilepsy, juvenile myoclonic epilepsy, and epilepsy with generalized tonic-clonic seizures alone. GGEs are mostly thought to be genetic epilepsies with a complex, polygenic inheritance.

Figure 1. Overview of main monogenic causes of epilepsy classified into generalized/multifocal versus focal phenotypes, and structural versus non-structural disorders.



ADPEAF, autosomal dominant partial epilepsy with auditory features; ADSHE, autosomal dominant sleep-related hypermotor epilepsy; BFIE, benign familial infantile epilepsy; **DS**, Dravet syndrome; **EFMR,** epilepsy and mental retardation limited to females; **EIEE**, early infantile epileptic encephalopathy; **EIMFS**, epilepsy of infancy with migrating focal seizures; **FCD**, focal cortical dysplasia; **FFEF**, familial focal epilepsy with variable foci; **FS**, febrile seizures; **GEFS+**, generalized epilepsy with febrile seizures+; **GGE**, genetic generalized epilepsy; **HH**, hypothalamus hamartoma; **HMEG**, hemimegalencephaly; **JAE**, juvenile absence epilepsy; **JME**, juvenile myoclonic epilepsy; **LEAT**, low-grade epilepsy-associated tumor; **MOGHE**, mild malformation of cortical development with oligodendroglial hyperplasia and epilepsy; **NF**, neurofibromatosis; **PMG**, polymicrogyria; **PNH**, periventricular nodular heterotopia; **SWS**, Sturge-Weber syndrome; **TSC**, tuberous sclerosis complex; **TLE**, temporal lobe epilepsy

[a] Secondary to hippocampal sclerosis/dual pathology
[b] Associated MCD has been (sporadically) described
[c] Multiple types of focal as well as generalized seizures
[d] Unclear if/how many non-operated MRI-negative patients have FCD

However, in family linkage studies, pathogenic variants in single genes associated with this phenotypic spectrum have been reported. Pathogenic variants in *GABRA1*, *CLCN2*, and *EFHC1* have been described in families with juvenile myoclonic epilepsy, but also in heterogeneous GGE phenotypes.[4,36] *GABRG2* and *CACNA1H* pathogenic mutations were identified in families with childhood absence epilepsy.[37] Other studies provided some evidence that *GABRD*, *ME2*, *BRD2*, and *NEDD4L* are susceptibility genes for GGE.[38-41] Recent genome-wide association studies have shown that GGE is indeed a multifactorial genetic disease, in which multiple susceptibility loci across the genome confer a low risk for disease.[42]

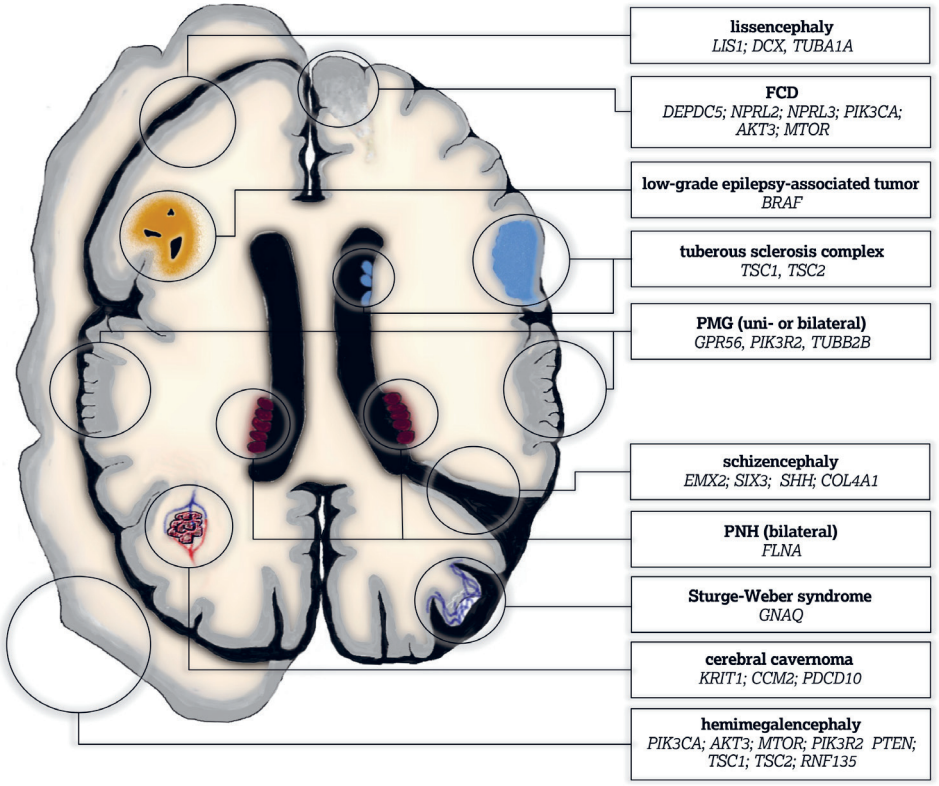
Structural epilepsies

Similar advances in epilepsy genetics, especially over the past two decades, have been made in identifying the genetic and molecular basis of structural epilepsies, which are clinically related to a focal or multifocal phenotype. Focal cortical dysplasias (FCDs), in particular FCD type II, are well-known underlying cortical malformations of severe pharmacoresistant epilepsies.[43] Important clinical syndromes predominantly, but not exclusively related to FCDs are ADSHE, familial focal epilepsy with variable foci, and ADPEAF. Although FCD is usually sporadic, family studies have identified germline or somatic single gene mutations in the mTOR pathway in many cases of FCD, in particular type II.[16] This pathway is involved in various developmental central nervous system processes such as neuronal growth, migration and proliferation. Upregulation of the mTOR pathway is also implicated in the pathogenesis of other neurological conditions associated with cortical malformations and intractable seizures, including TSC and hemimegalencephaly. The mTOR complex 1 (mTORC1) represents one of the most important regulators of cell growth within this pathway. GATOR1 (*DEPDC5*, *NPRL2*, *NPRL3*) as well as *TSC1* and *TSC2* are all negative regulators of the mTORC1 complex. Other key molecules, more upstream in this signaling pathway, are PI3K and AKT, and pathogenic variants in several genes related to these regulators (e.g., *PIK3CA*, *PIK3R2*, and *AKT3*) have been identified in focal epilepsy patients with various types of brain malformations (**figure 2**).[44]

The main hypothesis explaining the genetic basis of a focal structural etiology in general and the observed variation of lesions and phenotypic differences within families with the same pathogenic variant in particular is the so-called second-hit theory. This theory emphasizes that lesions could result from two different mutational events: one causing a germline variant in a cortical development gene carried within the family that is by itself not sufficient to cause disease, and the other causing a somatic mutation of the other allele of that gene expressed within the FCD (or other MCD). This hypothesis may explain why family members may remain asymptomatic, if a second hit does not occur. Furthermore, differences in lesion characteristics could be explained by the assumption that, between family members with the same pathogenic variant, the second mutation can affect neurodevelopmental precursors at different time points.[45]

Other important MCDs with an acknowledged genetic basis include diffuse neuronal migration disorders such as lissencephaly, periventricular heterotopia, and (mainly bilateral) polymicrogyria.[46] So far, more than 100 genes presumably associated with one or more types of MCD have been identified.[46] Aside from MCDs, other genetic structural disorders, increasingly recognized as neurodevelopmental diseases with focal or multifocal epilepsy as important clinical feature, are neurofibromatosis type I, cerebral cavernous malformations, and Sturge-Weber syndrome.[47]

Figure 2. Overview of important structural epilepsies associated with a genetic cause.



Challenges of genetics in epilepsy surgery

All efforts to identify epilepsy-causing genes and elucidate its neurobiological framework are driven by the goal of improving epilepsy care. The identification of a causative pathogenic variant could guide treatment choices, as well as prognostic and genetic counselling. Examples of so-called precision medicine for genetic epilepsies are the avoidance of sodium-channel blockers in Dravet syndrome (*SCN1A*), and the ketogenic diet as the major treatment for GLUT-1 deficiency (*SLC2A1*).[48] From the perspective of epilepsy surgery, the identification of epilepsy genes is being increasingly translated into precision medicine in specific cases. In general, epilepsy surgery is presumed to be the optimal treatment for refractory epilepsy patients with a focal, lesional epilepsy, implicating a well-defined epileptogenic zone that can be localized and delineated in order to enable complete resection or disconnection, provided it is located outside eloquent cortex. In contrast, patients with a primary genetic cause of epilepsy, implicating a more diffuse pathophysiological mechanism that cannot be translated into a well-defined removable epileptogenic zone, are presumed to be less eligible for resective surgery as well as for invasive diagnostics. Although there are several prognostic factors for postsurgical seizure outcome, selection of suitable candidates is still not optimal and exact indications and contraindications are not completely known.

In many patients, especially those without a visible structural cerebral abnormality (MRI-negative patients), extensive preoperative diagnostic work-up is required, including invasive intracranial electroencephalography monitoring in an increasing number of patients. Currently, on average only 65% of patients achieve seizure freedom after surgery, and operated MRI-negative patients have an even lower chance of reaching seizure freedom.[49]
Furthermore, the absence of a specific histopathological abnormality (approximately 8% of all operated patients) is a major predictor of poor outcome.[50] The crucial differentiation between patients with operable and non-operable epilepsy (i.e. between a presumed lesional and non-lesional cause of seizures) requires new and reliable biomarkers. Recent literature has shown that some of the pathogenic variants underlying epilepsy may be related to the effectiveness of epilepsy surgery in focal epilepsy patients. A systematic review, which included a total of 82 patients, showed that surgery was successful in terms of complete control of seizures in only 14% of patients with pathogenic variants in genes related to channel function and synaptic transmission (e.g., *SCN1A*, *SCN1B*, and *CNTNAP2*), despite focal semiology of at least some of the patient's seizures (**table 2** and **table 3**).[51]

In contrast, epilepsy surgery completely controlled seizures in 58% of patients with epilepsy due to germline mutations in the mTOR pathway (e.g., *DEPDC5*, *NPRL2*, and *NPRL3*). The success rate in this subgroup was 71 % for germline and somatic mutations combined.[51]
The disappointing overall seizure outcome of surgery in patients with pathogenic variants in genes related to channel function and synaptic transmission suggests a relative contraindication for epilepsy surgery (and invasive diagnostics), particularly in MRI-negative patients. An exception could be if epilepsy surgery is used as palliative treatment to target specific intractable focal seizures related to, for example, hippocampal sclerosis in *SCN1A*-related epilepsy, without the primary aim of achieving complete seizure freedom for all seizure types. On the other hand, the finding of a germline or mosaic mTOR gene pathogenic variant could justify continuation of the presurgical diagnostic process, even if a patient is MRI-negative. Such a finding could increase the chance of an underlying MCD being responsible for the patients' focal epilepsy. Although the low number of surgical cases for most genetic causes hampers firm conclusions, these findings encourage the evaluation of pathogenic variants in novel and known epilepsy genes as potential biomarkers to improve presurgical decision-making and ultimately to enhance postsurgical seizure outcome in patients with refractory epilepsy.
To date, genetic diagnostics are not routinely performed within the presurgical evaluation trajectory. In patients who undergo genetic testing, the genetic diagnosis is often established only after the patient has already been accepted or rejected for surgery. This could predispose patients to either undergo unnecessary invasive diagnostics or even resective surgery (e.g., if the pathogenic variant points toward a genetic syndrome without an identifiable lesion), or to be unjustly rejected for surgery (e.g., if the pathogenic variant points toward a structural epilepsy that is caused by an 'MRI-invisible' microstructural lesion). Larger and prospective studies are needed to further elucidate the importance of genetic testing in patients who are considered possible candidates for epilepsy surgery.

Table 2. Success rates of epilepsy surgery for patients with different genetic causes (germline mutations) of epilepsy.

genetic cause		MRI-lesional seizure-free/total	MRI-non-lesional seizure-free/total	total group seizure-free/total
gene mutations involved with channelopathies and disorders of synaptic transmission	*SCN1A*	FCD: 0/2 HS: 0/2 encephalomalacia: 0/1 subcortical area of abnormal signal: 0/1	0/2	0/8
	SCN1B	HS: 1/1	1/1	2/2
	CNTNAP2	HS: 0/2	0/1	0/3
	STXBP1	-	0/1	0/1
	overall	**1/9**	**1/5**	**2/14 (14%)**
mTOR pathway gene mutations	*DEPDC5*	FCD: 3/6	2/3	5/9
	PTEN	hemimegalencephaly: 1/1	-	1/1
	NPRL2	-	0/1	0/1
	NPRL3	FCD: 1/1	-	1/1
	overall	**5/8**	**2/4**	**7/12 (58%)**
other genetic causes of epilepsy	microdeletions	HS: 9/10	0/2	9/12
	neurofibromatosis type 1	FCD: 2/2 HS: 4/6 polymicrogyria 0/1 tumour 5/11	1/1	12/21
	fragile-x syndrome	HS: 2/2	-	2/2
	mitochondrial mutations	HS: 1/3	-	1/3
	overall	**23/35**	**1/3**	**24/38 (63%)**
total		**29/52 (56%)**	**4/12 (33%)**	**33/64 (52%)**

Table 3. Success rates of epilepsy surgery for patients with different genetic causes (somatic mutations) of epilepsy. FCD: focal cortical dysplasia; HS: hippocampal sclerosis (reproduced from Stevelink et al., 2018).[51]

		MRI-lesional seizure-free/total	**MRI-non-lesional seizure-free/total**	**total group seizure-free/total**
mTOR pathway gene mutations	*PIK3CA*	hemimegalencephaly: 5/5 FCD: 1/1	-	6/6
	AKT3	hemimegalencephaly: 1/3 FCD: 1/1	-	2/4
	MTOR	hemimegalencephaly: 1/1 FCD: 6/7	-	7/8
total		**15/18 (83%)**	**-**	**15/18 (83%)**

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3

Epilepsy surgery for patients with genetic refractory epilepsy: a systematic review

Remi Stevelink, Maurits W.C.B. Sanders, Maarten P. Tuinman, Eva H. Brilstra, Bobby P.C. Koeleman, Floor E. Jansen, Kees P.J. Braun

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ABSTRACT

Objectives

In recent years, many different DNA mutations underlying the development of refractory epilepsy have been discovered. However, genetic diagnostics are still not routinely performed during presurgical evaluation and reports on epilepsy surgery outcomes in patients with genetic refractory epilepsy are limited. We aimed to create an overview of the literature on seizure outcome following epilepsy surgery in patients with different genetic causes of refractory epilepsy.

Methods

We systematically searched PubMed and Embase prior to January 2017 and included studies describing treatment outcome following epilepsy surgery in patients with genetic causes of epilepsy. We excluded studies in which patients were described with epilepsy due to Tuberous Sclerosis Complex or Sturge-Weber syndrome (since this extensive body of research has recently been described elsewhere) and articles in which surgery was aimed to be palliative.

Results

We identified 24 eligible articles, comprising a total of 82 patients who had undergone surgery for (mainly childhood-onset) refractory epilepsy due to 15 different underlying genetic causes. The success rate of surgery varied widely across these different genetic causes. Surgery was almost never effective in patients with epilepsy due to mutations in genes involved in channel function and synaptic transmission, whereas surgery was significantly more successful regarding seizure control in patients with epilepsy due to mutations in the mTOR pathway. Patients with a lesion on MRI tended to have higher seizure freedom rates than those who were MRI-negative.

Conclusion

Although the evidence is still scarce, this systematic review suggests that studying genetic variations in patients with refractory epilepsy could help guide the selection of surgical candidates.

Introduction

It is estimated that around 60% of epilepsy patients have focal epilepsy, of whom nearly half are medically refractory.[1] Epilepsy surgery is the only treatment that may be curative in patients with medically refractory epilepsy. However, epilepsy surgery is strongly under-utilised and currently less than half of refractory epilepsy patients are referred for evaluation of epilepsy surgery candidacy.[2,3]

The relatively low proportion of potential surgical candidates who actually undergo surgery is largely due to a lack of factual information regarding epilepsy surgery and uncertainty around treatment outcome.[4] Although there are several prognostic factors for surgical success,[1] it is often unclear for which patients surgery is indicated or contraindicated. Currently, on average, only 65% of patients achieve seizure freedom after surgery.[1]

Over recent years, it has increasingly been acknowledged that many patients with either generalised or focal types of epilepsy have an underlying genetic cause.[5,6] These include single gene mutations that are related to channelopathies and disorders of synaptic transmission,[5] or the mammalian target of rapamycin (mTOR) pathway, involved in various processes such as neuronal growth, migration, and proliferation.[7] In addition, there are several microdeletions and other chromosomal abnormalities that are known to be associated with epilepsy. This heterogeneity in molecular genetic aetiology reflects the underlying pathophysiological diversity and explains the phenotypic variation among patients. It is possible that these different causes are also associated with differences in response to epilepsy surgery.

It is commonly accepted that epilepsy patients with a genetically determined focal structural lesion(s), such as tuberous sclerosis, may be candidates for surgery.[8] However, many patients with genetic causes of epilepsy do not have detectable epileptogenic lesions on MRI, so called 'MRI-negative' patients. In general, the absence of a visible brain lesion on MRI significantly decreases the chance of surgical success.[9,10] MRI-negative patients with focal epilepsy can still be considered surgical candidates as there may be an undetected underlying focal epileptogenic brain lesion, such as mild malformations of cortical development (mMCD) or focal cortical dysplasia (FCD).[11,12] Greater MR field strength, improved MRI sequences, and new post-processing techniques have increased the detection rate of such mMCDs and FCDs.[12] Even in truly MRI-negative patients with refractory focal epilepsy and a consistent electrophysiological focus, epilepsy surgery is increasingly considered due to advances in multimodal functional neuroimaging and invasive monitoring techniques, such as stereo-electroencephalography (S-EEG). Pathology often subsequently reveals an underlying mMCD or FCD.[12] However, surgery has been successful in some (18-47%) patients without demonstrated pathological abnormalities.[9] A still unknown proportion of MRI-negative patients with focal refractory epilepsy who are evaluated for epilepsy surgery may have a genetic epilepsy syndrome. Identification of such genetic causes could have prognostic value for surgical outcome in these patients.

Genetic diagnostics are still not routinely performed in patients with refractory epilepsy, mostly due to the high costs and low throughput of traditional DNA sequencing techniques.[6] The possibility to comprehensively test all epilepsy patients for genetic causes has recently improved with the advent of next-generation sequencing techniques.[6]

To date, reports of epilepsy surgery for patients with genetic causes of epilepsy are sporadic. Some recent studies have shown that epilepsy surgery may be effective in patients with mutations in specific genes but this has never been shown in patients with other gene mutations.[13-16] Such findings suggest that routine genetic diagnostics for causative mutations of epilepsy prior to surgery could be of importance to determine surgical candidacy. This systematic review provides an over-

view of the reported outcomes of epilepsy surgery in patients with an established genetic cause of epilepsy. Future aims include the use of genetic diagnostics in the presurgical assessment of patients with refractory epilepsy in order to assist the clinician in the often complex dilemma of whether to proceed to surgery or rather stop the time-consuming, costly, and often invasive, presurgical trajectory in patients with a proven genetic epilepsy syndrome.

Methods

Search strategy and study selection

Our search strategy and study selection are summarised in **figure 1**. A literature search in PubMed and Embase was performed by one author (RS) in order to identify articles in which epilepsy, genetics, and surgery were described together, using various synonyms (**supplementary tables 1** and **2**). The search was initially performed in June 2016 and updated in November 2016. The search yielded a total of 1,345 articles. We included all studies reporting on epilepsy surgery and seizure outcome and collected details only on patients who either had a definite clinical diagnosis of a genetic syndrome with co-morbid epilepsy, or who had a mutation or other genetic abnormality detected that was highly likely to be the cause of their epilepsy. All patients with genetic causes of epilepsy who were described in the reports were included, regardless whether the causative mutation was somatic/mosaic or germline, although we describe the results for these subgroups separately. We excluded articles on patients with epilepsy due to tuberous sclerosis complex or Sturge-Weber syndrome from this systematic review, since this extensive body of research has recently been described elsewhere.[17,8] Furthermore, we excluded articles in which epilepsy surgery was described for patients with genetic mutations that were associated with, but not considered monogenic causes of, their epilepsy; for example, BRAF mutations in glioneuronal tumours, reported as potential prognostic factors for surgery outcome.[18] Moreover, we excluded surgical cases in which the intention of surgery was stated to be palliative rather than curative.

All search results were reviewed based on title and abstract. The full-text was reviewed in potentially eligible articles. Moreover, references of the included articles were reviewed, as well as other articles in which the eligible articles are cited, using the 'cited by' functions in PubMed and Embase. The article search and selection were checked by a second author (MS).

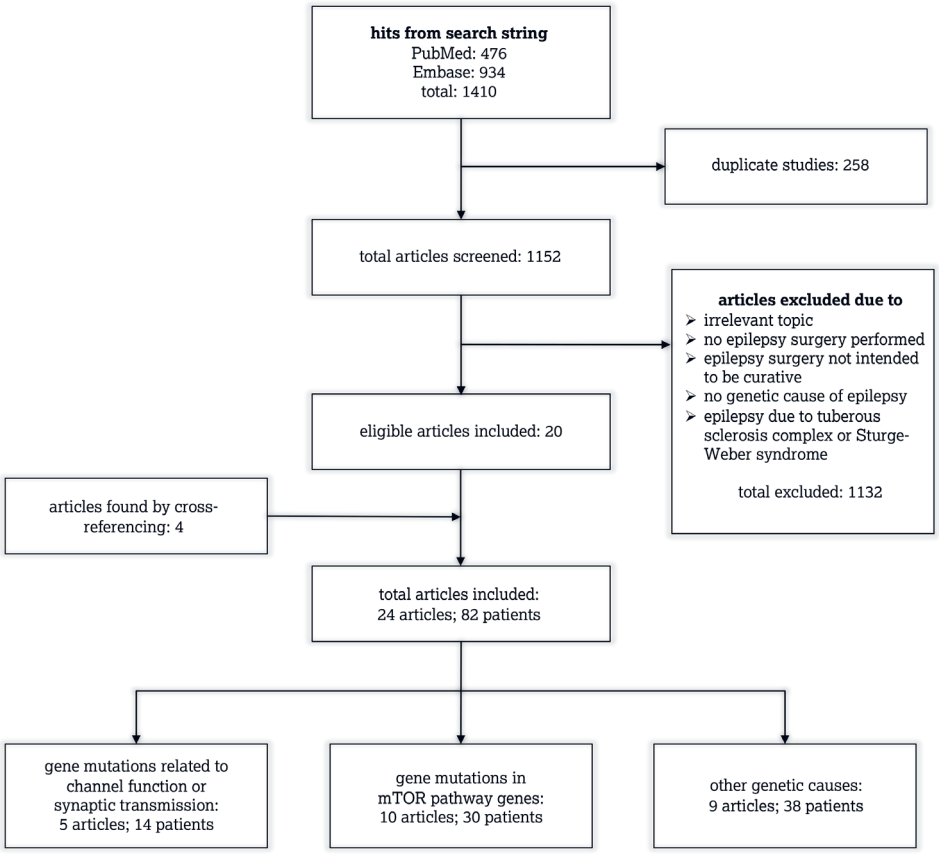
Data processing

A standardised data extraction form was created, containing nine variables: affected gene, causative genetic variants, number of patients, histology of resected tissue, MRI findings, surgery type, mean follow-up time in years, postsurgical seizure outcome, and whether the surgery was successful. We divided the included articles into three main categories of genetic causes of epilepsy:
pathogenic variants of genes related to ion channel function and synaptic transmission;
pathogenic variants of mTOR-pathway genes; other genetic causes of epilepsy.

Extraction of raw data from the included articles was performed by RS and checked by MS.

Where possible, we classified histological descriptions of resected or isolated tissue according to the standardised classification system of focal cortical dysplasia (FCD) defined by the ILAE. MRI findings where catogerised as FCD, hippocampal sclerosis (HS) or hemimegalencephaly when applicable. Patients were defined as MRI-negative based on either no abnormalities or only non-specific abnormalities, not judged to be the cause of epilepsy, on MRI. All patients without detect-

Figure 1. Flowchart of search strategy and study selection.



able causative lesions on MRI were used for subgroup analysis. Successful surgery was defined as Engel Class I ('free of disabling seizures'), the equivalent ILAE class 1, or a description of seizure outcome equivalent to these classifications, based on the last reported follow-up visit.

Results

Search results

The literature search yielded a total of 20 eligible articles and an additional four publications were identified through a cross-reference check of the citations of the included articles, as well as all publications in which the eligible articles are cited.

The 24 included studies described a total of 82 patients, with 15 different genetic causes of (mainly childhood-onset) epilepsy, who underwent surgery. The success rate of surgery varied widely among these different genetic causes (**table 1**).

Table 1a. Success rates of epilepsy surgery for patients with different genetic causes (germline mutations) of epilepsy.

genetic cause		MRI-positive seizure-free/total	MRI-negative seizure-free/total	total group seizure-free/total
gene mutations associated with channelopathies and disorders of synaptic transmission	*SCN1A*	FCD: 0/2 HS: 0/2 encephalomalacia: 0/1 subcortical area of abnormal signal: 0/1	0/2	0/8
	SCN1B	HS: 1/1	1/1	2/2
	CNTNAP2	HS: 0/2	0/1	0/3
	STXBP1	-	0/1	0/1
	overall	**1/9**	**1/5**	**2/14 (14%)**
mTOR pathway gene mutations	*DEPDC5*	FCD: 3/6	2/3	5/9
	PTEN	HMEG: 1/1	-	1/1
	NPRL2	-	0/1	0/1
	NPRL3	FCD: 1/1	-	1/1
	overall	**5/8**	**2/4**	**7/12 (58%)**
other genetic causes of epilepsy	microdeletions	HS: 9/10	0/2	9/12
	neurofibromatosis type 1	FCD: 2/2 HS: 4/6 polymicrogyria: 0/1 tumour: 5/11	1/1	12/21
	fragile-x syndrome	HS: 2/2	-	2/2
	mitochondrial mutations	HS: 1/3	-	1/3
	overall	**23/35**	**1/3**	**24/38 (63%)**
total		**29/52 (56%)**	**4/12 (33%)**	**33/64(52%)**

FCD: focal cortical dysplasia; HS: hippocampal sclerosis. HMEG: hemimegalencephaly.

Table 1b. Success rates of epilepsy surgery for patients with different genetic causes (somatic mutations) of epilepsy.

genetic cause		MRI-positive seizure-free/total	MRI-negative seizure-free/total	total group seizure-free/total
mTOR pathway gene mutations	*PIK3CA*	HMEG: 5/5 FCD: 1/1	-	6/6
	AKT3	HMEG: 1/3 FCD: 1/1	-	2/4
	MTOR	HMEG: 1/1 FCD: 6/7	-	7/8
total		**15/18 (83%)**	**-**	**15/18 (83%)**

FCD: focal cortical dysplasia; HMEG: hemimegalencephaly.

Genes related to channel function and synaptic transmission

The literature search yielded five articles that described a total of 14 surgery cases with epilepsy due to pathogenic variants in genes related to ion channel function and synaptic transmission (**table 2**). These epileptogenic mutations were found in the voltage-gated sodium channels *SCN1A* and *SCN1B*,[5] the gene *CNTNAP2* which is involved in AMPA-receptor trafficking and excitatory neuronal network activity,[19-20] and *STXBP1*, which is involved in the release of neurotransmitters.[21] Epilepsy surgery did not lead to complete seizure freedom in any of the eight patients with *SCN1A* mutations who underwent epilepsy surgery, even though six of them had focal seizure semiology which co-localised with MRI-visible lesions.[15-16] Outcome data concerning specific seizure types that were primarily targeted by the surgical procedure (e.g. temporal lobe seizures in patients with HS) were not provided in the publications included. Seven of the patients with *SCN1A* mutation had a clinical phenotype consistent with Dravet syndrome, and one had a clinical phenotype most consistent with genetic epilepsy with febrile seizures plus (GEFS+). Two patients had no MRI-visible lesion.

Two patients underwent surgery for epilepsy due to mutations in *SCN1B* and both patients became seizure-free after temporal lobectomy;[22] one had underlying HS, whereas no brain abnormality, on MRI or histopathological examination of resected tissue, was detected in the other patient. All three patients with epilepsy due to a homozygous mutation in *CNTNAP2* had a recurrence of seizures after surgery.[23] One patient with epilepsy due to a *STBXP1* mutation underwent surgery since she had prominent focal findings on EEG, despite having no abnormalities on MRI. Epilepsy surgery did not lead to cessation of seizures although her seizure frequency had decreased.[21] Pathology of the resected tissue revealed FCD.

Overall, surgery was successful regarding the control of seizures for only two of 14 patients (14%) with pathogenic variants in genes related to channelopathies and disorders of synaptic transmission.

mTOR pathway genes

The search yielded 10 articles that described a total of 30 patients who underwent surgery for epilepsy in relation to mutations in the following mTOR pathway genes: *DEPDC5*, *PTEN*, *PIK3CA*, *AKT3*, *NPRL2*, *NPRL3*, and *MTOR* itself (**table 3**). In 12 patients, germline mutations were found in *DEPDC5*, *PTEN*, *NPRL2* or *NPRL3* genes, whereas in 18 patients (somatic or mosaic) mutations were detected in resected tissue, involving the genes PIK3CA, AKT3, and mTOR. Epilepsy surgery controlled seizures completely in seven of 12 patients with mutations in *DEPDC5*, *PTEN*, *NPRL2* or *NPRL3*, of whom eight had a lesion on MRI.[14,24-27] Three more patients had a significant improvement in seizure frequency, whereas two patients had no improvement. Fifteen of 18 patients with somatic or mosaic mutations in *PIK3CA*, *AKT3* or *MTOR*, who were all reported to have lesions on MRI, became seizure-free after epilepsy surgery.[13,14,28-31] One patient reported some improvement, in another monthly seizures persisted, and the last patient did not become seizure-free, however, outcome was not further specified. After correlating histology with MRI findings, 19 of the 30 patients (somatic/mosaic or germline combined) had focal cortical dysplasia (FCD) due to mTOR pathway pathogenic variants as a structural substrate of epilepsy, whereas 10 other patients had hemimegalencephaly as the structural cause of their epilepsy. One patient had normal MRI and histology. Epilepsy surgery successfully controlled seizures in eight of the 10 patients with hemimegalencephaly (80%) and in 14 of the 19 patients with FCD (74%). Epilepsy surgery was not successful for the patient with normal MRI and histology.

Overall, epilepsy surgery completely controlled seizures in seven of 12 patients (58%) with epilepsy due to germline mutations in the mTOR pathway. The success rate was 71% (22 of 30 patients) for germline and somatic mutations combined.

Epilepsy due to other genetic causes

Eleven articles described a total of 38 patients (all but three with MRI-visible lesions) who had epilepsy in relation to the following other genetic causes: microdeletions, neurofibromatosis type 1, fragile-X syndrome, and mitochondrial mutations (**table 4**). Twelve patients who underwent epilepsy surgery have been reported with microdeletions, four of which were identified in 16p13.11.[32-33] Nine of 12 patients (75%) became seizure- free after surgery; one patient remained seizure-free for seven years after surgery, and the other two patients experienced no improvement. Twenty-one patients with neurofibromatosis type 1, caused by mutations in *NF1* or microdeletions in 17q11.2 encompassing this gene, underwent epilepsy surgery.[34-36] These patients had a variety of neurofibromatosis-related epileptogenic lesions, such as HS or low-grade tumours. Epilepsy surgery success- fully controlled seizures in 12 of 21 patients (57%) with neurofibromatosis type 1. Two patients with epilepsy due to Fragile-X syndrome, both with HS, became seizure-free after epilepsy surgery.[37-38] Three patients with epilepsy and mitochondrial mutations, who all had HS (detected on MRI), underwent epilepsy surgery; only one became seizure-free.[39-40]

MRI-negative patients with genetic epilepsy

A subgroup analysis of all MRI-negative patients with genetic causes of epilepsy yielded a total of 12 patients with mutations (all detected in blood, and not in tissue) in *SCN1A*, *SCN1B*, *CNTNAP2*, *STXBP1*, *DEPDC5*, and *NPRL2*, and microdeletions in 16p13.11, or neurofibromatosis type 1 (**table 1** and **table 5**). Five MRI-negative patients had epilepsy due to mutations in genes related to channelopathies and disorders of synaptic transmission.[16,21-23] According to the reports, surgery was considered in these patients based on focal seizure semiology in combination with consistent

EEG source localization and results from functional imaging, EEG or S-EEG results.[16,21-23] Surgery did not successfully control seizures in any of these patients, except in one with a mutation in *SCN1B*. Surgery was performed in four MRI-negative patients who had epilepsy due to mutations in the mTOR pathway genes, *DEPDC5* or *NPRL2*, and showed focal abnormalities on EEG, S-EEG or PET.[24,25,27] Two MRI-negative patients with *DEPDC5* mutations showed FCD on pathological examination and became seizure-free after surgery, whereas surgery did not successfully control seizures in one patient with a pathology-negative *DEPDC5* mutation and another with a mutation in *NPRL2* and FCD on pathological examination. Epilepsy surgery did not successfully control seizures in either of the two MRI-negative patients with epilepsy due to a microdeletion in 16p13.11. These patients underwent surgery for clinically presumed HS, although neither had HS on pathological examination.[32,33] One MRI-negative patient with epilepsy due to neurofibromatosis type 1, with focal abnormalities in the temporal region on EEG and pathology revealing HS in resected tissue, underwent epilepsy surgery and subsequently became seizure-free.[34] After histological examination, five of the 12 MRI-negative patients were shown to have features of FCD (type Ia, IIa, or not further specified), one patient had HS, and another had a small epileptogenic hamartoma. Five of the 12 MRI-negative patients (42%) had no abnormalities on histological examination.

Overall, a seizure freedom rate of 33% (4 of 12 patients) was reported in the MRI-negative group; two with a mutation in a mTOR pathway gene, one with a *SCN1B* mutation, and one with an *NF1* mutation. Histological examination showed a lesion in three of these patients but no abnormality in the patient with the *SCN1B* mutation. One of the five MRI-negative patients without histopathological abnormalities achieved seizure freedom after epilepsy surgery, whereas three of seven MRI-negative patients with histopathological abnormalities became seizure-free.

Statistical analyses

Surgery was more successful for patients with mTOR pathway mutations, compared to patients with mutations in genes involved in channelopathies and disorders of synaptic transmission (only patients with germline mutations: 58% versus 14%; χ^2=5.54; df=1; p=0.019; germline and somatic mutations combined: 73% versus 14%; χ^2=13.42; df=1; p<0.001; only patients with MRI-visible lesions: 77% versus 11%; χ^2= 12.07; df=1; p<0.001). The difference in surgery success rate between patients with MRI-visible lesions and MRI-negative patients was at trend level (63% versus 33%, χ^2=3.679; df=1; p=0.055).

Table 2. Seizure outcome for patients with epilepsy due to mutations in genes involved in channelopathies and disorders of synaptic transmission.

study	gene	pathogenic variants	number of patients	histology of epileptic focus	MRI findings	surgery type	postsurgical seizure outcome	successful epilepsy surgery	mean follow-up time in years (range)
Barba et al. **2014**	*SCN1A*	c.317C>T; c.2584C>G	2	FCD type Ia (1/2); FCD type IIa (1/2)	FCD (2/2)	right temporal lobectomy and parieto-occipital corticectomy	Engel Class IV (1/2), postoperative death (1/2)	0/2	not reported
Skjei et al. **2015**	*SCN1A*	c.2927del; c.5434T>G; c.4587del; c.5018T>G; c.1661A>G; deletion at exons 17-20	6	mild MCD (4/6); non-specific findings, possibly due to sampling errors (2/6)	HS (2/6); haemorrhage and encephalomalacia after injury (1/6); small area of abnormal signal (1/6); MRI-negative or non-specific (2/6)	frontal lobectomy (2/6); temporal lobectomy and frontal gyrus resection (1/6); focal lesionectomy (3/6)	ILAE Class 5 (5/6) ILAE Class 4 (1/6)	0/6	3.15 (1.5 - 5.9)
Scheffer et al. **2007**	*SCN1B*	c.363C>G	2	HS (1/2); non-specific findings (1/2)	HS (1/2); non-specific (1/2)	temporal lobectomy	seizure-free (2/2)	2/2	2.5 (2 - 3)
Strauss et al. **2006**	*CNTNAP2*	3709delG	3	diffuse dysplasia (3/3)	temporal lobe abnormalities suggestive of MTS (2/3), MRI-negative (1/3)	temporal lobectomy (1/3), amygdalohippocampectomy (1/3) and limited cortical resection (1/3)	all patients had a recurrence of seizures from 6 to 15 months after surgery	0/3	not reported

Weckhuysen et al. 2013 2013	*STXBP1*	c.1631G>T	1	FCD type IA	normal	complete occipital disconnection and multiple subpial transections	95% reduction in seizure frequency (equivalent to Engel Class III)	0/1	not reported

FCD: focal cortical dysplasia; MCD: malformations of cortical development; MRI; magnetic resonance imaging; HS: hippocampal sclerosis; N/A: not applicable; ILAE Class: International League Against Epilepsy classification for seizure outcome following epilepsy surgery.

Table 3. Seizure outcome after epilepsy surgery in patients with epileptogenic mutations in mTOR pathway genes.

study	gene	pathogenic variants	number of patients	histology of epileptic focus	MRI findings	surgery type	postsurgical seizure outcome	successful epilepsy surgery	mean follow-up time in years (range)
Baulac et al. **2015**	*DEPDC5*	c.715C>T; c.4841G>A; c.1264C>T; c.1759C>T	5	FCD type I (1/5); type IIa (2/5); not conclusive due to fragmented specimen (2/5)	FCD (3/5); MRI-negative (2/5)	local resection at different sites	seizure-free (3/5), worthwhile improvement (1/5) no improvement (1/5)	3/5	8 (4 - 13)
Carvill et al. **2015**	*DEPDC5*	c.3092C>A; c.842A>T	2	Normal (1/2); FCD type IIa (1/2)	normal (1/2); FCD (1/2)	temporal corticectomy (1/2); lobectomy followed by functional hemispherectomy (1/2)	no improvement (1/2) and seizure-free for 2.5 years, after which monthly staring spells (1/2)	0/2	not reported
Scerri et al. **2015**	*DEPDC5*	c.1663C>T	2	FCD type IIa (2/2)	FCD (2/2)	hemispherectomy (1/2); local resection (1/2)	seizure-free (2/2)	2/2	13 (10 - 16)
Jansen et al. **2015**	*PTEN*	p.Tyr68His (germline)	1	HMEG and FCD	HMEG	hemispherectomy	seizure-free	1/1	not reported
	PIK3CA	p.His1047Arg (1/2), p.Glu545Lys as well as p.Thr544Asn (1/2); all mosaic mutations	2	HMEG (1/2), FCD type IIa (1/2)	HMEG (1/2); FCD (1/2)	hemispherectomy (1/2) and occipital resection (1/2)	seizure-free	2/2	not reported
Conti et al. **2015**	*AKT3*	mosaic trisomy of the 1q21.1-q44	1	FCD type Ib	FCD	lesionectomy	seizure-free, except when withdrawing medication	1/1	3

Lee et al. **2012**	*PIK3CA*	somatic p.Glu54Lys mutation	4	HMEG and FCD (4/4)	HMEG (4/4)	hemispherectomy	seizure-free (4/4)	4/4	not reported
	AKT3	somatic p.Glu17Lys mutation	1	HMEG and FCD	HMEG	hemispherectomy	not seizure-free	0/1	not reported
	MTOR	somatic p.Cys1483Tyr mutation	1	HMEG and FCD	HMEG	hemispherectomy	seizure-free	1/1	not reported
Nakashima and Saitsu, 2015	*MTOR*	somatic p.Ser2215Tyr (2/6); p.Ala1459Asp; p.Leu1460Pro; p.Ser2215Phe; p.Leu1460Pro mutations	6	FCD (6/6)	FCD (6/6)	lesionectomy (5/6); amygdalohippocampectomy and temporal lobectomy (1/6)	seizure-free (5/6); monthly seizures (1/6)	5/6	not reported
Poduri et al. **2012**	*AKT3*	somatic p.E17K activating mutation in AKT3 (1/2) and mosaic trisomy of chromosome 1q involving AKT3 (1/2)	2	HMEG and FCD (2/2)	HMEG (2/2)	hemispherectomy	seizure-free (1/2); reduction to 1-4 per month (1/2)	1/2	5.5 (5 - 6)
Leventer et al. **2015**	*MTOR*	mosaic c.4487T>G mutation	1	FCD type IIa	FCD	hemispherectomy followed by removal of right frontobasal connection	seizure-free	1/1	not reported
Weckhuysen et al. **2016**	*NPRL2*	C68_69delCT	1	FCD type Ia	normal	fronto-orbital resection	50% seizure reduction	0/1	not reported
	NPRL3	c.1270C>T	1	FCD type IIa	FCD and hippocampal atrophy	lesionectomy for fcd, twice due to incomplete resection	rare seizures when medication errors	1/1	not reported

FCD: focal cortical dysplasia; MRI: magnetic resonance imaging; HMEG: hemimegalencephaly.

Table 4. Seizure outcome after epilepsy surgery in patients with epilepsy due to other genetic causes.

study	**genetic cause/ syndrome**	**number of patients**	**histology of epileptic focus**	**MRI findings**	**surgery type**	**postsurgical seizure outcome**	**successful epilepsy surgery**	**mean follow-up time in years (range)**
Catarino et al. **2011**	microdeletions in: 16p13.11 (3/10); 17p12 (2/10); 15q11.2 (2/10); 4q32.3 (1/10; 4q35.2 (1/10); 7q31.32-31-33	10	HS (8/10); hamartoma (1/10); non-specific findings (1/10)	HS (7/10), hippocampal atrophy (1/10), hippocampal asymmetry (1/10) and MRI-negative (1/10)	anterior temporal lobectomy (7/10); amygdalo-hippocampectomy (2/10); neocorticectomy and amygdalectomy (1/10)	ILAE Class 1 (8/10); ILAE Class 5 (1/10); ILAE Class 1 for 7 years then ILAE Class 3 (1/10)	8/10	4.9 (1 - 13)
Liu et al. **2012**	microdeletions in 16p13.11	2	HS (1/2) and hamartoma in white matter of middle temporal gyrus (1/2)	hippocampal atrophy (1/2) and MRI-negative (1/2)	hippocampal and temporal cortical resection (2/2)	seizure-free (1/2) and still experiencing frequent seizures (1/2)	1/2	not reported
Barba et al. **2013**	neurofibromatosis type 1	12	DNET tumour (5/12); HS (4/12); polymicrogyria (1/12); mixed pathology (1/12); no pathology (1/12)	HS (3/12); cystic lesion (3/12); cortical dysplasia (2/12); hyperintensities (2/12); polymicrogyria (1/12); MRI-negative (1/12)	lobectomy (7/12), lesionectomy (1/12), corticectomy (1/12), cortico+lesionectomy (2/12), focal resection+disconnection (1/12)	Engel Class Ia (8/12), Engel Class IV (4/12)	8/12	2.6 (1 - 10)
Ostendorf et al. **2013**	neurofibromatosis type 1	8	HS (2/8); astrocytoma (3/8); glioma (1/8); DNET tumour (1/8); epidermoid (1/8)	MRI and histology not separated in paper	resection (5/8); lobectomy (3/8)	Engel Class Ia (3/8), Engel Class IIb (1/8), Engel Class IV (4/8)	3/8	7.4 (1 - 33)
Jang et al**. 2013**	neurofibromatosis type 1	1	HS	HS	anteromesial temporal resection	seizure-free	1/1	not reported

Wouters et al. **2006**	fragile-x syndrome: expansion of CGG repeats in *FMR1* gene	1	HS	HS	temporal lobectomy	seizure-free	1/1	“to date”
Kenmuir et al. **2015**	fragile-x syndrome: expansion of CGG repeats in *FMR1* gene	1	HS	HS	left anterior temporal lobectomy	seizure-free	1/1	1
Niehusmann et al. **2011**	mitochondrial mutations in ND2 (1/2) and ND4 (1/2)	2	HS (1/2); non-specific (1/2)	HS (2/2)	amygdalohippocampectomy (1/2) and lesionectomy, sparing hippocampus and amygdala (1/2)	Engel Class III (1/2); Engel Class IIIa (1/2)	0/2	not reported
Azakli et al. **2013**	mitochondrial mutation in *ND1*	1	HS	HS	amygdalohippocampectomy	seizure-free 3 years after surgery	1/1	3

FCD: focal cortical dysplasia; DNET: dysembryoplastic neuroepithelial tumour; HS: hippocampal sclerosis; N/A: not applicable; ILAE Class: International League Against Epilepsy classification for seizure outcome following epilepsy surgery.

Table 5. Seizure outcome after epilepsy surgery in the subgroup of MRI-negative patients with genetic epilepsy.

study	gene	pathogenic variants	number of patients	histology of epileptic focus	MRI findings	surgery type	postsurgical seizure outcome	successful epilepsy surgery	mean follow-up time in years (range)
Skjei et al. **2015**	*SCN1A*	c.2927del, c.1661A>G	2	no gross abnormalities (2/2); increased cell numbers in 1 patient	non-specific (2/2)	frontal lobectomy (1/2) and focal lesionectomy (1/2)	ILAE Class 5 (1/2); ILAE Class 4 (1/2)	0/2	3.4 (3 - 4)
Scheffer et al. **2007**	*SCN1B*	C121W	1	non-specific findings	non-specific scattered and diffuse white matter hyperintensities	anterior temporal lobectomy	seizure-free	1/1	2
Strauss et al. **2006**	*CNTNAP2*	3709delG	1	FCD	no dysplasia	temporal lobectomy, amygdalohippocampectomy and limited cortical resection	Recurrence of seizures started somewhere between 6 to 15 months after surgery (not further specified)	0/1	not reported
Weckhuysen et al. **2013**	*STXBP1*	c.1631G>T	1	FCD type Ia	normal	complete occipital disconnection and multiple subpial transections	95% reduction in seizure frequency	0/1	not reported
Baulac et al. **2015**	*DEPDC5*	c.484-1G>A (1/2); c.715C>T and c.1264C>T (1/2)	2	FCD type I (1/2); FCD type IIa (1/2)	normal	local resection	seizure-free (2/2)	2/2	13

Carvill et al. **2015**	*DEPDC5*	c.3092C>A	1	normal	normal	temporal corticectomy	no improvement	0/1	not reported
Weckhuysen et al. **2016**	*NPRL2*	C68_69delCT	1	FCD type Ia	normal	fronto-orbital resection	50% seizure reduction	0/1	not reported
Catarino et al. **2011**	16p13.11	chr16:15387380-16198600 microdeletion	1	non-specific findings	normal	neocorticectomy and amygdalectomy	ILAE 1 for 7 years then ILAE 3	0/1	13
Liu et al. **2012**	16p13.11	*NDE1*-containing microdeletion in resected tissue	1	hamartoma in subcortical white matter	normal	hippocampal and temporal cortical resection	still experiencing frequent seizures	0/1	not reported
Barba et al. **2013**	*NF1*	17q11.2 microdeletion (Neurofibromatosis type 1)	1	HS	normal	temporal lobectomy	Engel Class Ia	1/1	2

FCD: focal cortical dysplasia; HS: hippocampal sclerosis; ILAE Class:
International League Against Epilepsy classification for seizure outcome following epilepsy surgery.

Discussion

In this systematic review, we provide an overview of the reported seizure outcomes of patients with different genetic causes of refractory epilepsy who have undergone epilepsy surgery. Not unexpectedly, there was a large difference in the success rate of epilepsy surgery between patients with mutations in genes related to channelopathies and disorders of synaptic transmission and those with mutations in the mTOR pathway, even when somatic mutations were excluded from analysis. This difference remains significant when only MRI-positive cases are compared. mTOR pathway genetic variants are thought to increase seizure susceptibility due to abnormal neuronal migration and growth, which leads to (micro)structural epileptogenic malformations of cortical development, such as hemimegalencephaly and FCD.[14] Such malformations are thought to arise from a combination of a germline mTOR pathway mutation and a somatic second-hit mutation in the same gene or in a different gene of the mTOR pathway.[24,41] This typically results in focal malformations, since the second hit usually only affects part of the brain. It is reasonable to assume that resection of such localised epileptogenic malformations could be a curative treatment for seizures, as reflected by the relatively high surgical success rate of patients with mTOR pathway mutations. It has been estimated that 11% of all focal epilepsies are due to germline mutations in the mTOR genes *DEPDC5*, *NPRL2* and *NPRL3*.[27] Considering the associated high success rate of epilepsy surgery, it could be of benefit to routinely screen for such mutations in presurgical evaluation; particularly in MRI-negative, but presumed lesional cases. Finding mTOR pathway mutations would increase the chance of identifying an underlying cryptic malformation of cortical development, and thereby suggest surgical candidacy. The high success rate (83%) of surgery in patients with somatic/mosaic mTOR pathway gene mutations is inherent to the fact that these patients already had established epileptogenic lesions (FCD and hemimegalencephaly); two factors associated with good surgical outcome. Screening for somatic/mosaic mutations in presurgical evaluation is more difficult than for germline mutations. However, investigation of mosaic mutations may be considered in samples of blood, a buccal swab, or sputum using ultra-deep sequencing.[42]

Epilepsy surgery was almost never successful in patients with epilepsy due to mutations in genes involved in channelopathies and disorders of synaptic transmission. Germline mutations in these genes involved in ion channel function and synaptic transmission are likely to cause widespread aberrant neuronal activity,[5] which is rarely confined to a specific part of the brain. It is unlikely that a local resection would be curative to prevent all seizure types. Surgery did not lead to seizure freedom for any reported patient with mutations in *SCN1A*, *CNTNAP2* or *STXBP1* in this series, despite focal semiology for (at least some of their) seizures, and the fact that most of the patients had coincident structural (possibly) epileptogenic lesions. It is likely that these lesions were either not directly related to the genetic cause of epilepsy or that the lesions accounted for only some of the seizures. Possibly, surgery in these patients was not aimed at curing all seizure types, but only targeted seizures originating from a specific structural lesion. However, such a goal was not specified in any of the included articles, nor was the selective outcome for these specific "targeted" seizures. The disappointing overall seizure outcomes of surgery in patients with mutations in this group of genes suggest a relative contraindication for epilepsy surgery, particularly in MRI-negative patients.

Surgery successfully controlled seizures in both patients with mutations in *SCN1B*, one of whom was MRI-negative. In mice, one of the two splice variants of *Scn1b* is known to encode a secreted cell adhesion molecule involved in neuronal pathfinding during embryonic development, and epileptogenic mutations in *Scn1b* result in a functional knockout of this splice variant.[43] Moreover, *Scn1b* knockout mice exhibit defective neuronal proliferation and migration in the hippocampus, which precedes

hyperexcitability.[44] These findings suggest that *SCN1B* mutations may be associated with structural epileptogenic abnormalities, and focal resection may thus lead to favourable surgical outcome, rather than directly influencing neuronal excitability, as is the case for *SCN1A* mutations.[5]

We found large differences in the success rate of surgery for epilepsy due to other genetic causes. Epilepsy surgery effectively controlled seizures in most described patients with epilepsy-associated microdeletions. Most of these patients, however, had HS as an underlying structural epileptogenic substrate, which is generally associated with a favourable surgical outcome. Epilepsy surgery was effective in more than half of the neurofibromatosis type 1 patients. Similar to the situation in patients with pathogenic variants in mTOR pathway genes, *NF1* is thought to affect only those parts of the brain with a second-hit mutation,[41] which could explain why resection of these affected parts can be curative. Epilepsy surgery for patients with Fragile-X syndrome or mitochondrial mutations could effectively control seizures, although only a few patients have been described.

Based on a subgroup analysis, we examined whether epilepsy surgery could be effective for MRI-negative patients with genetic epilepsy. Interestingly, MRI-negative patients had a wide range of different genetic causes (**table 1**), but surgical success rate tended to be higher in cases with MRI showing visible lesions (66%) than in MRI-negative cases (33%), which is in line with previous studies.[9] Interestingly, two patients were reported after successful epilepsy surgery for genetic refractory epilepsy, but histological examination of the resected tissue did not reveal any abnormalities. However, we cannot exclude the possibility that subtle abnormalities may have gone undetected due to sampling errors, or that the resection may have removed crucial parts of the epileptogenic non-lesional network. The outcome of these patients suggests that the absence of a detectable lesion on MRI in patients with genetic abnormalities should not in itself be an absolute contraindication for epilepsy surgery.

It remains unclear whether structural lesions are truly absent in MRI-negative patients, or whether their apparent absence is simply based on limitations such as the detection sensitivity threshold of MRI performed or the experience of the radiologist.[12] In accordance with previous studies.[9,12] we found that most MRI-negative patients who underwent surgery in this review had histological abnormalities suggestive of MCD in the resected tissue. New MRI methods, higher-field scanning, and post-processing techniques have already shown that it is possible to detect epileptogenic lesions which were not previously visible on conventional MRI scans, improving the identification of surgical target areas and subsequently yielding higher success rates in patients with genetic refractory epilepsy.

There are a number of limitations to this systematic review. The low number of surgical cases for most genetic causes hampers firm conclusions. Furthermore, there is significant heterogeneity between reported patient characteristics and surgical procedures. The follow-up duration largely varies between studies and is sometimes not reported. Another source of heterogeneity stems from different mutations within the same gene among patients, which could potentially affect surgical outcome. Moreover, differences of expertise in genetic analysis or surgery, accessibility to genetic testing, and indications for epilepsy surgery could relate to lower reporting and different success rates of surgery. Although not explicitly stated in most studies, we assumed that reported mutations were detected in blood, unless specified otherwise. The extent of mosaicism and the effect on the occurrence of a lesion and surgical outcome remains unclear. In addition, publication bias, recall bias, and selection bias due to the scarce number of patients described in the literature cannot be excluded; it is possible that unsuccessful surgery is less likely to be reported.

Surgical candidacy, particularly for MRI-negative patients, is still not easily determined. Some patients are declined surgery because of a presumed non-structural, genetic aetiology. Finding a

germline or mosaic mTOR gene mutation could justify continuation of the presurgical diagnostic process. Others, however, are offered resective surgery or invasive monitoring (S-EEG) (because of presumed focal structural aetiology despite negative MRI), although their epilepsy may have been primarily caused by a genetic and more diffuse aetiology, such as mutations in genes involved in ion channel or neurotransmitter function. Genetic testing is not yet routinely included in most surgical evaluation programmes. Nevertheless, finding specific gene mutations could prove valuable for the process of selecting surgical candidates and counseling patients on expected outcome. Larger and prospective studies are needed to further elucidate the importance of detecting genetic mutations in patients who are considered possible candidates for epilepsy surgery.

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Supplemental information

Summary didactic slides are provided online with the published version of this chapter: https://onlinelibrary.wiley.com/doi/10.1684/epd.2018.0959

Supplementary table 1: PubMed search string. The search was performed on 8 July 2016.

search term	synonyms
epilepsy	Epilepsy[MeSH] OR epilepsy[tiab] OR epileptic[tiab]
genetic	"Mutation"[MeSH] OR "Mutation/genetics"[MeSH] OR "Drug Resistant Epilepsy/genetics"[MeSH] OR genetic[tiab] OR mutation[tiab] OR mutations[tiab] OR familial[tiab] OR hereditary[tiab] OR "autosomal dominant"[tiab] OR "copy number variant"[tiab] OR "copy number variation"[tiab] OR CNV[tiab] OR microdeletions[tiab] OR "single-nucleotide polymorphism"[tiab] OR Chromosomal[tiab] OR Rett[tiab] OR Angelman[tiab] OR Pitt-Hopkins[tiab] OR Mowat-Wilson[tiab] OR POLG[tiab] OR CDG[tiab] OR "Congenital disorder of glycosylation"[tiab] OR fragile-X[tiab] OR ADNFLE[tiab] OR "Autosomal dominant lateral temporal epilepsy"[tiab] OR ADLTE[tiab] OR "ring 20"[tiab] OR "ring chromosome 20"[tiab] OR "r(20) syndrome"[tiab]
Ssurgery	"Drug Resistant Epilepsy/surgery"[MeSH] OR surgery[tiab] OR resection[tiab] OR surgical[tiab] OR lesionectomy[tiab] OR operati*[tiab]

Subsequent rows were linked with AND.

Supplementary table 2: Embase search string. The search was performed on 8 July 2016.

search term	synonyms
epilepsy	'epilepsy'/exp OR epilepsy:ti,ab; epileptic:ab,ti
genetic	'mutation'/exp OR 'genetic disorder'/exp OR genetic:ti,ab OR mutation:ti,ab OR mutations:ti,ab OR familial:ti,ab OR hereditary:ti,ab OR 'autosomal dominant':ti,ab OR 'copy number variant':ti,ab OR 'copy number variation':ti,ab OR CNV:ti,ab OR microdeletions:ti,ab OR 'single-nucleotide polymorphism':ti,ab OR Chromosomal:ti,ab OR Rett:ti,ab OR Angelman:ti,ab OR Pitt-Hopkins:ti,ab OR Mowat-Wilson:ti,ab OR POLG:ti,ab OR CDG:ti,ab OR "Congenital disorder of glycosylation":ti,ab OR fragile-X:ti,ab OR ADNFLE:ti,ab OR "Autosomal dominant lateral temporal epilepsy":ti,ab OR ADLTE:ti,ab OR "ring 20":ti,ab OR "ring chromosome 20":ti,ab OR "r(20) syndrome":ti,ab
surgery	'neurosurgery'/exp OR surgery:ti,ab OR resection:ti,ab OR surgical:ti,ab OR lesionectomy:ti,ab OR operati*:ti,ab

Subsequent rows were linked with AND. Publications were filtered on the publication type 'Article'.

4

Implications of genetic diagnostics in epilepsy surgery candidates: a single-center cohort study

Maurits W.C.B. Sanders, Cynthia M.C. Lemmens, Floor E. Jansen, Eva H. Brilstra, Bobby P.C. Koeleman, Kees P.J. Braun

on behalf of the Dutch Collaborative Epilepsy Surgery Programme (LWEC)

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ABSTRACT

Objective

Genetic causes are increasingly identified in patients with focal epilepsy. These genetic causes may be related to the effectiveness of epilepsy surgery. We aimed to assess the use and yield of genetic testing in a large cohort of patients who were evaluated for epilepsy surgery.

Methods

We performed a retrospective single-center consecutive cohort study of patients who were evaluated for surgery between 1990 to 2016. Within this cohort, we assessed the use of genetic testing, either before or after presurgical decision-making. We evaluated genetic results as well as the outcome of presurgical decision-making and surgery, and compared these endpoints for different subgroups (especially MRI-positive ***versus*** MRI-negative patients). Patients with tuberous sclerosis (TSC) and ***KRIT1*** mutations were excluded from analysis.

Results

Of the 2385 epilepsy patients who were evaluated for surgery, 1280 (54%) received surgical treatment in our center. Of the entire cohort, 325 (14%) underwent genetic testing, comprising 156 of 450 MRI-negative patients (35%) ***versus*** 169 of 1935 MRI-positive patients (9%). A genetic cause of epilepsy was found in 40 of the 325 patients (12%, 2% of the entire cohort), mainly consisting of mutations in ion channel function and synaptic transmission genes, and mTOR pathway gene mutations.
Three of the 7 patients with mTOR pathway gene mutations underwent surgery; two achieved complete seizure-freedom. One of the 17 patients with germline mutations in ion channel function and synaptic transmission genes received resective surgery but was not rendered seizure-free; 2 other patients underwent invasive intracranial EEG-monitoring before being rejected.

Significance

This study shows that genetic testing is increasingly applied in focal epilepsy patients who are considered for epilepsy surgery. The diagnostic yield of genetic testing is highest in next-generation sequencing techniques, and the outcome of genetic testing assists selecting eligible patients for invasive intracranial monitoring and resective surgery.

Introduction

Epilepsy is one of the most common neurological diseases globally, with a lifetime prevalence of 7.6 in 1,000 people.[1] Up to one-third of patients develop pharmacoresistance, and many have significant comorbidities, such as developmental delay.[2–4]

In patients with pharmacoresistant focal epilepsy originating from a structural abnormality, resective surgery represents the only potentially curative treatment option provided that the lesion can be accurately delineated and is located outside eloquent cortex. In many patients, especially those without a structural abnormality, extensive preoperative diagnostic work-up is required, including invasive intracranial EEG-monitoring in an increasing number of patients.[4] Despite rapidly improving presurgical diagnostic modalities and increasing clinical experience, patient selection for surgical treatment is still suboptimal, with on average only 65–69% of patients achieving postoperative seizure-freedom.[6,7] Therefore, new and reliable markers to properly differentiate between patients with operable and non-operable epilepsy are warranted.

In the last decades, novel epilepsy genes have been increasingly identified in patients with generalized as well as focal epilepsy.[8] The possibilities to comprehensively test epilepsy patients for a genetic etiology have expanded throughout the last years, with the advent of readily available next generation sequencing techniques (NGS).[9] In particular whole exome sequencing (WES), through which the coding sequence of all genes in the human genome can be scanned for apparent disease-causing sequence variation, accelerated the discovery of novel epilepsy genes in the past decade.[10] The importance of genetic causes of structural focal epilepsies was first established by the observation that inherited or de novo mutations in genes involved in the mTOR pathway, can cause focal epilepsy.[11] It stands without a doubt that the identification of a causative mutation could affect treatment as well as prognostic and genetic counseling (Reif et al, 2016).[12] Moreover, recent literature shows that some of the epilepsy gene mutations may also be related to the effectiveness of epilepsy surgery in focal epilepsy patients (Stevelink et al, 2018).[13] We therefore hypothesize that genetic testing can add valuable information to current preoperative patient assessment.

To gain more insight in the evolution of diagnostic genetic testing in epilepsy surgery evaluation, we studied the application and yield of genetic testing within a large consecutive cohort of epilepsy patients who were evaluated for epilepsy surgery in a tertiary referral center over the past decades. We assessed differences in outcome between important subgroups of evaluated patients, especially focusing on MRI-negative *versus* MRI-positive patients.

Methods

Patient selection

Patients were identified within the database of the Dutch Collaborative Epilepsy Surgery Program group, coordinated in the UMC Utrecht. We included all children and adults who were consecutively evaluated for epilepsy surgery from January 1st, 1990 to January 1st, 2017. We reviewed medical records, discharge letters and outpatient clinical summaries of all identified patients. Subjects who did not complete the presurgical program (i.e. patient self-withdrawal), and those in whom the UMC Utrecht was not the operating theater were excluded from this analysis. Moreover, we excluded patients if adequate medical information on presurgical assessment could not be retrieved from the medical files. We separately report patients with tuberous sclerosis complex (TSC) and multiple cavernoma (due to *KRIT1* or other CCM mutations) in this cohort, because these concern specific groups of patients in whom the diagnosis is made on the basis of the pathognomonic

imaging (MRI) findings in combination with clinical criteria. Genetic testing in patients with TSC and multiple cavernoma is mainly performed to confirm the diagnosis and to facilitate genetic counseling. In addition, an extensive body of research to evaluate the role of epilepsy surgery in TSC has already been described elsewhere,[14,15] and genetic testing is not likely to be related to surgical success.

If patients were evaluated multiple times, solely the most recent episode of preoperative assessment was included as timepoint of surgical decision-making. In patients who underwent reoperation for failed epilepsy surgery, we only assessed data on follow-up after the last surgery.

The medical ethics committee of the UMC Utrecht approved the study and concluded that no informed consent was required for this retrospective observational study on available data from routine clinical care.

Data collection

We collected information on the results of all structural and functional imaging and genetic diagnostic modalities that were utilized within the presurgical evaluation trajectory of included patients. Results of brain MRI as reported by dedicated epilepsy neuroradiologists and the multidisciplinary team discussions at the time of presurgical evaluation were recorded for each of the patients. Scans were not re-reviewed for the purpose of this study. In patients who underwent genetic diagnostics, results of each separate genetic test were collected. We evaluated the results of all clinical genetic tests performed, regardless of whether the genetic results became available before, during or after the presurgical trajectory.

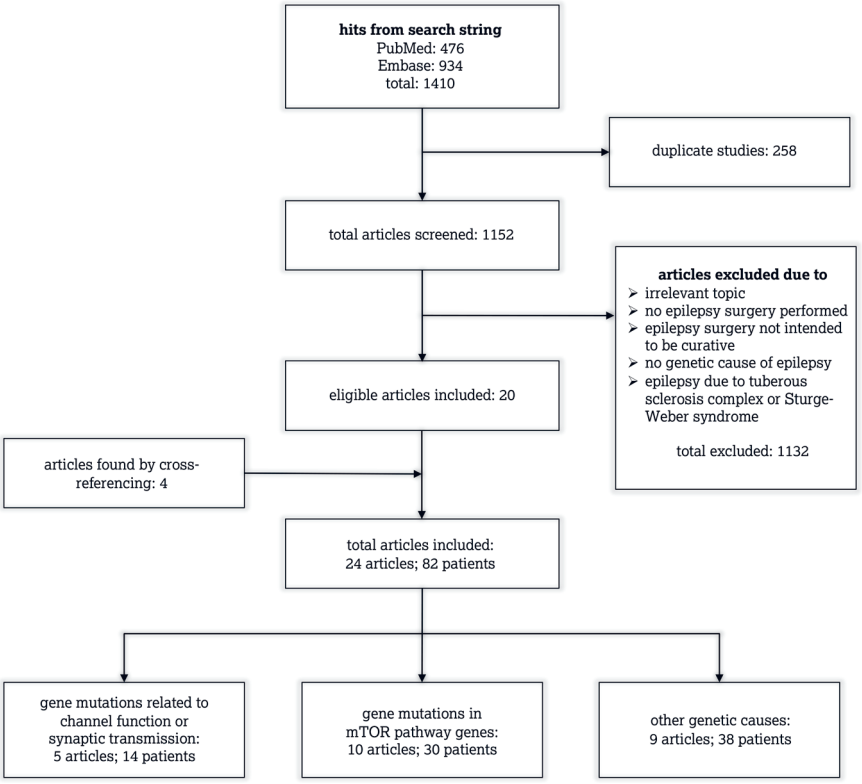
We classified the genetic tests into six categories according to the different technologies that were used: 1) karyotype analysis, 2) array techniques (e.g. SNP-array or array-CGH), and sequencing techniques consisting of 3) individual gene analysis, 4) targeted gene panels (categorized into 10 panels; examples are: focal epilepsy, fever-sensitive epilepsy or epileptic encephalopathy), 5) full epilepsy gene panel (including >130 epilepsy genes up to >170 in recent years) and 6) whole exome sequencing (WES). All tests were performed on blood samples and reported causal variants concern germline mutations. Reported genetic variants and clinical information were reviewed by two authors: EB, an experienced clinical geneticist, and MS. Variants were classified into five categories, (according to the American College of Medical Genetics and Genomics criteria) that convey information about the probability of representing a pathogenic variant that is causative for the patients' epilepsy.[16] These categories comprised i) benign ii) likely benign iii) uncertain significance, iv) likely pathogenic and v) pathogenic. For the analysis of this study, we considered solely class iv (likely pathogenic) and v (pathogenic) variants to be causative for the epilepsy. We assessed the pathogenic nature of variants with use of common predictors for pathogenicity such as segregation analysis, functional analysis, appearance of the mutation in variant-related control databases and molecular features of the mutation itself.

If epilepsy surgery was performed, we extracted information on type of surgery, histopathological diagnosis and postoperative seizure outcome from the patients' clinical files. Seizure outcomes at 1 year of follow-up after surgery was classified according to the Engel classification system; we defined seizure-freedom as complete cessation of seizures –including aura's– (Engel 1A).[17]

Results

Medical information was retrieved for 2385 people with epilepsy (1022 children; 43%) who completed the presurgical evaluation program within the UMC Utrecht (**figure 1**). Of these, 450 (19%) had no reported MRI abnormalities causative for the epilepsy ('MRI-negative'), whereas 1935 subjects (81%) were classified as 'MRI-positive', with a lesion considered epileptogenic. Complete postoperative seizure-freedom (Engel 1A) in all operated patients (n=1280) occurred significantly more often in MRI-positive patients (74%) compared to the MRI-negative patients (49%, p = <0.001).

Figure 1: Enrollment of study patients.



In total, 662 genetic tests were performed in 325 of all patients (14%). MRI-negative patients were significantly more likely to undergo genetic testing than patients who had a causative MRI-lesion (35% *versus* 9%, OR 3.9; 95% CI 3.2–4.7). Outcome of surgical evaluation with respect to different subgroups of patients is listed in **table 1**. Overall, 181 of 325 patients (56%) underwent genetic testing with results available before completion of the presurgical evaluation trajectory, whereas in the remaining 44% genetic testing was performed only after the patient was already accepted or rejected for surgery. Fifteen of the 40 subjects (38%) in whom a genetic diagnosis could be established underwent genetic analysis only after surgical decision-making.

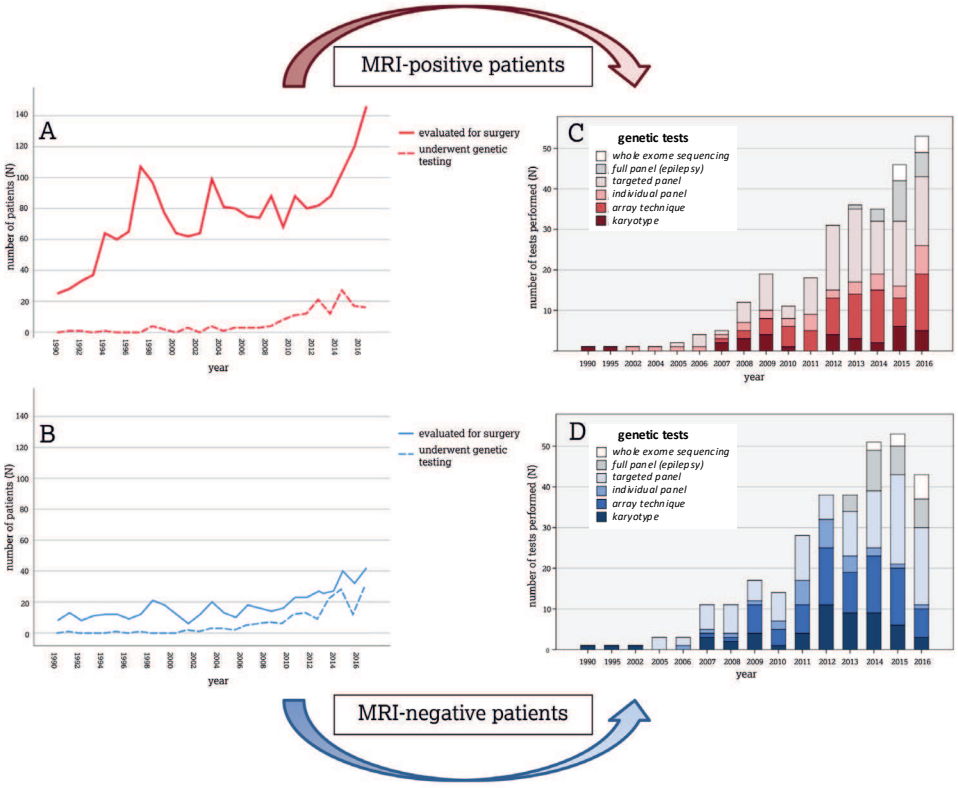
Table 1. Outcome of surgical evaluation with respect to different subgroups of patients.

I: COHORT		II: SURGICAL DECISION	III: SURGICAL OUTCOME
subgroup	**N**	**accepted for surgery N** *(%)*	**seizure-free N** *(%)*
all patients	2385	1280 *(54)*	863/1209[a] *(71)*
MRI-negative	450	119 (*26)*	52/106 *(49)*
MRI-positive	1935	1161 *(60)*	811/1103 *(74)*
genetic testing	325	113 *(35)*	49 *(43)*
MRI-negative	156	34 *(22)*	9 *(26)*
MRI-positive	169	79 *(47)*	40 *(51)*
genetic diagnosis	40	13 *(30)*	5 *(38)*
MRI-negative	25	2 *(8)*	1 *(50)*
MRI-positive	15	9 *(60)*	3 *(33)*
mTOR gene mutations	7	3 *(42)*	2 *(67)*
channel function and synaptic transmission gene mutations	17	1 *(6)*	0 *(0)*

[a] exact data on surgery outcome could not be retrieved in 74 of 1283 cases

The annual number of patients evaluated for epilepsy surgery gradually increased from 33 (8 MRI-negative; 25 MRI-positive) in 1990 to 188 (42 MRI-negative; 146 MRI-positive) in 2016 (**figure 2a–b**). The percentage of patients undergoing genetic testing increased from 3% in 1991 to an average of 30% of all evaluated patients in the past 5 years. In 1991, 13% of MRI-negative patients *versus* 0% of the MRI-positive group underwent genetic testing, and, over the past 5 years, genetic testing was performed in 69% of MRI-negative patients *versus* 20% of the MRI-positive population. Genetic testing performed between 1990 and 2013 resulted in 13 of the 43 (30%) genetic diagnoses, whereas the remaining 30 (70%) genetic causes were established from 2013 onwards.

Figure 2a–d. Annual (1990–2016) number of patients who underwent i) presurgical assessment (unbroken line) and ii) genetic testing (broken line; a, b) and quantity and type of genetic tests performed (c, d). Red color: MRI-positive patients; blue color: MRI-negative patients.



Karyotyping and array techniques were among the most commonly used types of genetic testing from 1990 to 2010, whereas from 2005 onwards, NGS techniques were also used more widely (**figure 2c–d**). Together with full epilepsy gene panels and WES, which were first used within this cohort in 2013 and 2014 respectively, these multiple gene sequencing techniques are currently the most widely used as well as efficient techniques for mutation screening in patients evaluated for epilepsy surgery.

Genetic testing yielded an abnormal finding (i.e. genetic variant) in 144 of all patients (44%) tested, comprising 77 of 156 MRI-negative patients (49%) *versus* 67 of 169 MRI-positive (40%) patients. A (likely) pathogenic variant was found in 40 of the 325 tested patients, and did not significantly differ between MRI-negative and MRI-positive patients (20 *versus* 20 patients respectively, p = 0,404). As expected, the diagnostic yield of multiple gene sequencing (NGS) techniques was significantly higher (p = <0.001) compared to other tests. The diagnostic yield with respect to finding disease-causing variants was lowest for array techniques (2%) and individual gene analysis (2%) and highest for full epilepsy panel sequencing (17%) and whole exome sequencing (24%). The diagnostic yield of different types of genetic testing is listed in **table 2**.

Table 2. Outcome (III) and diagnostic yield (disease-causing variants) of genetic testing with regard to type (I) and number (II) of genetic tests performed.

I: TYPE OF TESTING	II: FREQUENCY OF TESTING	III: FINDINGS	
genetic test	**number of tests N**	**any genetic variant N** *(%)*	**causative genetic variant N** *(%)*
karyotype analysis	91	3 *(3)*	3 *(3)*
array technique	165	47 *(28)*	4 *(2)*
individual gene sequencing	56	4 *(7)*	1 *(2)*
targeted panel sequencing[1]	257	39 *(15)*	14 *(5)*
febrile-related epilepsy	91	19 (21)	6 (7)
focal epilepsy	42	8 (19)	3 (7)
epileptic encephalopathy	49	7 (14)	3 (6)
progressive myoclonic epilepsy	10	1 (10)	1 (10)
benign neonatal convulsions	21	1 (5)	0 (0)
epilepsy and mental retardation	37	3 (8)	2 (5)
idiopathic generalized epilepsy	3	0 (0)	0 (0)
paroxysmal disorders	3	0 (0)	0 (0)
inflammation mediated epilepsy	1	0 (0)	0 (0)
full epilepsy panel sequencing	64	34 *(53)*	11 *(17)*
whole exome sequencing	29	17 *(58)*	7 *(24)*
total	**662**	**144** (22)	**40** (6)

[1] panels as listed below included a number of genes that varied over time; more recent panels contained more genes. Furthermore, some genes were included in several panels.

Outcome in patients with an established genetic diagnosis

Three of the 7 patients with mTOR pathway gene mutations (2 *DEPDC5*; 1 *NPRL3*) underwent surgery (**table 3**). Two of these (one MRI-negative) achieved complete seizure-freedom. The third (MRI-negative) operated patient was not rendered seizure-free after surgery; the surgical report noted a tailored, possibly incomplete, resection of the epileptogenic zone in an eloquent (left precentral) area. Histopathological examination of the resected tissue in this patient was not indicative for a focal cortical dysplasia (FCD), nor did it reveal any other abnormalities. Three of the 4 patients with mTOR pathway gene mutations who were rejected for surgery were MRI-negative, and in 2 of these 4 (1 MRI-negative; 1 MRI-positive) the genetic diagnosis was established only after rejection for surgery.

Of the 17 patients (12 being MRI-negative) with germline mutations in ion channel function and synaptic transmission genes, only one (*CNTNAP2*) was accepted for epilepsy surgery. It concerned a patient in whom the genetic diagnosis was established preoperatively, with a subtle right temporal MRI-lesion that was consistent with the patient's semiology and ictal EEG findings. The patient underwent a focal resection and histopathological examination of resected tissue showed gliosis and was not indicative for any specific abnormalities. Five months after surgery the seizures recurred. Two other patients had undergone intracranial EEG-monitoring, and were rejected for resective surgery. In both, genetic results became available only months after the presurgical evaluation was completed. Overall, in 10 of the 17 patients (59%) with germline mutations in ion channel function and synaptic transmission, the genetic etiology was established only after rejection for surgery.

Seven of the 13 patients with epilepsy due to genetic causes other than mTOR pathway gene mutations or mutations in ion channel function and synaptic transmission genes received surgery. This group comprised 2 patients with a *NF1* pathogenic variant, 1 patient with epilepsy due to a 1q44 deletion (MRI showed unilateral polymicrogyria); 1 patient with a 12p13.33 deletion (MRI and histopathological examination indicative for hippocampal sclerosis); 1 patient with an *ARX* mutation (MRI showed unilateral polymicrogyria); 1 patient with an *ANKRD11* pathogenic variant (KBG-syndrome, MRI suggestive for FCD whereas histopathological examination was unremarkable); and 1 patient with a *WDR26* pathogenic variant (MRI showed hemiatrophy). Complete seizure cessation was achieved in 3 of these 10 patients. All 3 patients with ring chromosome 20 were rejected for surgery.

Patients with tuberous sclerosis complex and *KRIT1* mutations

There were 53 patients evaluated for surgery in whom a clinicoradiological diagnosis of TSC was established. Individual gene analysis in these patients resulted in a genetic diagnosis (*TSC1* or *TSC2* mutation) in 45 patients. In all but two patients, the genetic diagnosis was already known before the patients entered the epilepsy surgery evaluation program.

Three patients were found with epilepsy due to cavernomas in the context of a *KRIT1* pathogenic variant. Two of these patients were seizure-free (Engel 1A) one year after surgery.

Table 3. Timing of genetic analysis (pre-*versus* postsurgical decision-making) and surgical outcome (seizure-freedom) in patients with a pathogenic variant.

neurobiological pathway	**pathogenic variant (N)**	**genetic testing**							
	N=40 (total)	**before surgical decision-making (N=22)**				**after surgical decision-making (N=18)**			
		operated (N=9) **-** (seizure-free: N=3)		rejected (N=13)		operated (N=2) **–** (seizure-free: N=1)		rejected (N=16)	
		iEEG	no iEEG	iEEG (0)	no iEEG	iEEG	no iEEG	iEEG	no iEEG
mTOR pathway (n=7, 18%)	*DEPDC5* ***(5)***	1 (0)	-	-	2	1 (1)	-	-	1
	NPRL3 ***(2)***	-	1 (1)	-	-	-	-	-	1
Ion channel function and synaptic transmission (n=17, 43%)	*SCN1A* ***(6)***	-	-	-	3	-	-	2	1
	KCNT1 ***(3)***	-	-	-	-	-	-	-	3
	GRIN2A ***(2)***	-	-	-	1	-	-	-	1
	STXBP1 ***(2)***	-	-	-	-	-	-	-	2
	SLC35A2 ***(1)***	-	-	-	1	-	-	-	-
	CHRNA4 ***(1)***	-	-	-	1	-	-	-	-
	GABRA3 ***(1)***	-	-	-	-	-	-	-	1
	CNTNAP2 ***(1)***	-	1 (0)	-	-	-	-	-	-
cell-cell adhesion (n=1, 2%)	*PCDH19* ***(1)***	-	-	-	-	-	-	-	1
protein translation and modification (n=2, 5%)	*PNKP* ***(1)***	-	-	-	1	-	-	-	-
	QARS ***(1)***	-	-	-	1	-	-	-	-
neuronal migration, neurogenesis (n=1, 2%)	*ARX* ***(1)***	-	1 (1)	-	-	-	-	-	-
other causes (n=12, 30%)	microdeletions ***(4)***	-	2 (0)	-	1	-	-	-	1
	ring 20 ***(3)***	-	-	-	2	-	-	-	1
	NF1 ***(2)***	-	2 (1)	-	-	-	-	-	-
	ANKRD11 ***(1)***	-	1 (1)	-	-	-	-	-	-
	NBEA ***(1)***	-	-	-	-	-	-	-	1
	WDR26 ***(1)***	-	-	-	-	-	1 (0)	-	-

Discussion

In the past decades, there is a distinct increase in the application of genetic testing among patients with pharmacoresistant epilepsy who entered the Dutch comprehensive presurgical evaluation program, particularly in those with normal MRI. Furthermore, the spectrum of genetic technologies embedded in the presurgical assessment expanded clearly over the last 10 years, with a shift towards NGS technology. These two findings are parallel and were expected due to the rapid developments in the field of genetic technologies that improved clinically available and cost-effective genetic testing in epilepsy patients. The accelerated discovery of genes in human epilepsy together with the advent of readily available multiple gene sequencing techniques resulted into a significantly increased detection of disease-causing variants in our cohort. Our findings highlight the need for genetic testing in focal epilepsy in general and its potential use in epilepsy surgery evaluation specifically.

For the purpose of this study and to maintain a 'real-life situation', results of brain imaging as reported by experienced clinical neuroradiologists at the time of evaluation were not re-reviewed. It is commonly accepted that having a causative focal or unilateral structural abnormality on MRI (MRI-positive) is a strong predictor of good surgical outcome.[18] This finding was confirmed in our study, with 74% seizure-freedom in MRI-positive compared to 49% in MRI-negative patients. This emphasises the need for improved imaging or post-processing methods, and for new, non-invasive diagnostic modalities that could improve presurgical decision-making and ultimately surgical success.

In this study, 2 of 3 operated patients with mTOR pathway gene mutations achieved seizure-freedom (one of whom being MRI-negative) after surgery. These findings are in accordance with the results of our recent literature review, which showed that surgery in FE patients with mTOR pathway gene mutations –likely representing a focal, structural cause for epilepsy– had a relatively higher success rate compared to other genetic causes.[13] The characteristics of the MRI-negative third patient with no postoperative reduction of seizure frequency (small resection in an eloquent area and no pathology identified in resected tissue), point towards the possibility of an incomplete resection of the epileptogenic focus; the patient is therefore currently being re-evaluated. These three epilepsy patients are representative of the population in which a genetically established diagnosis may point to an 'MRI-invisible' microstructural lesion or malformation of cortical development, likely implying a reasonable chance of postoperative seizure freedom. Genetic testing may help differentiate this group from patients with a genetic syndrome not accompanied by an identifiable lesion, which likely reflects a lower chance of surgical success, or from patients with a combination of both.

On the other hand, genetic testing could also improve decision-making in MRI-positive patients. The identification of an mTOR gene mutation early in the presurgical evaluation could, together with other presurgical diagnostic modalities, add strength to the congruence of a structural localized epileptogenic focus.

The finding of a pathogenic variant indicating a genetic syndrome without a structural cause of epilepsy suggests poor surgical candidacy and outcome. Of the 14 patients reported in the literature with a mutation in genes related to ion channel or synaptic function, only 2 patients (14%) became seizure-free after surgery.[13] Of our 17 patients with such mutations, 16 patients were rejected because presurgical assessment failed to localize the epileptogenic zone. One MRI-positive patient (*CNTNAP2* mutation) received surgery and was not rendered seizure-free; clinical history and surgical outcome of this patient was in accordance with the few previously reported in the literature.[19] Two patients were rejected after they underwent invasive monitoring. In these 2 patients, a *SCN1A*

mutation was only found after the invasive monitoring had been performed. These findings suggest that identification of a pathogenic variant in this group of genes (before or during the presurgical evaluation) could prevent unnecessary extensive and expensive presurgical evaluation, and especially resective surgery or invasive source localization techniques in MRI-negative as well as MRI-positive patients with an epilepsy phenotype not fully in concordance with the lesion. In the latter group however, surgery may be considered to only target lesion related seizures in patients with dual pathology (e.g. patients with *SCN1A* mutations and hippocampal sclerosis).

In conclusion, there is a clear increase in the use of genetic testing in patients evaluated for epilepsy surgery, which can be explained by a rapid evolution of genetic techniques, the extensive discovery of novel generalized as well as focal epilepsy genes in the last decade, and the increase in number of MRI-negative patients, in whom both structural and non-structural genetic aetiologies are increasingly considered in the differential diagnosis.

However, genetic diagnostics are still not routinely performed before deciding to operate patients or reject them from surgery or from invasive monitoring. In many proposed flow diagrams of presurgical evaluation, genetic testing is not listed, and, in the recent ILAE evaluation of presurgical test utility, genetic tests were not included.[20,21] Moreover, in almost half of the patients who underwent genetic testing, the genetic diagnosis was established only after the patient was already accepted or rejected for surgery.

The low number of surgical cases for most genetic causes and the heterogeneity of pathogenic variants leave open questions. Therefore, a prospective study is warranted to identify causative epilepsy gene variants in patients who are evaluated for surgery, that can either point towards structural focal and presumably surgically remedial causes of epilepsy, or towards primary genetic non-lesional causes of epilepsy. We are currently undertaking this study with the hypothesis that genetic testing in patients with pharmacoresistant epilepsy can improve differentiation between good surgical candidates, and those who are less eligible for epilepsy surgery.

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Supplemental information

Supplementary table 1. Individual characteristics of genetic testing (detailed), imaging, surgical outcome and pathology (if accepted for surgery) of epilepsy patients with an established genetic cause.

pathway	pathogenic variant (n=40)	MRI	iEEG	surgery	pathology	FU surgery 1y
mTOR pathway	1. *DEPDC5* c.435G>A (p.Trp145*)	negative	-	-	-	-
	2. *DEPDC5* c.3994C>T (p.Arg1332*)	negative	grid	resection	normal	not SF
	3. *DEPDC5* c.4520-2A>G (p.?)	negative	-	-	-	-
	4. *DEPDC5* c.1310del (p.Asn437fs)	negative	-	-	-	-
	5. *DEPDC5* c.1310del (p.Asn437fs)	negative	grid	resection/disconnection	FCD IC	SF
	6. *NPRL3* c.348_351del (p.Leu117fs)	FCD	-	resection	FCD IIA	SF
	7. *NPRL3* c.83delT (p.Phe28fs)	FCD	-	-	-	-
ion channel function and synaptic transmission	8. *SCN1A* c.1178G>A (p.Arg393His)	cavernoma	-	-	-	-
	9. *SCN1A*: c.1719C>A (p.Ser573Arg)	negative	grid	-	-	-
	10. *SCN1A* c.4942C>T (p.Arg1648Cys)	negative	-	-	-	-
	11. *SCN1A* c.2945T>C (p.Val982Ala)	negative	-	-	-	-
	12. *SCN1A*[a]	negative	-	-	-	-
	13. *SCN1A*: c.4331C>A (p.Ser1444Tyr)	negative	grid	-	-	-
	14. *KCNT1* c.2882G>A (p. Arg961His)	negative	-	-	-	-
	15. *KCNT1* c.2782C>T (p.Arg928Cys)	negative	-	-	-	-
	16. *KCNT1* c.2882G>A (p.Arg961His)	negative	-	-	-	-

pathway	pathogenic variant (n=40)	MRI	iEEG	surgery	pathology	FU surgery 1y
ion channel function and synaptic transmission	17. *GRIN2A* c.2253dup (p.Ser752fs)	negative	-	-	-	-
	18. *GRIN2A* c.1613C>G (p.Ser538*)	heterotopia	-	-	-	-
	19. *STXBP1* c.785A>T (p.Asp262Val)	negative	-	-	-	-
	20. *STXBP1* c.1651C>T (p.Arg551Cys)	negative	-	-	-	-
	21. *SLC35A2*[a]	FCD	-	-	-	-
	22. *CHRNA4* c.851C>T (p.Ser284Leu)	negative	-	-	-	-
	23. *GABRA3* c.1421A>G (p.Tyr474Cys)	negative	-	-	-	-
	24. *CNTNAP2* c.1898-1_1903del (p.?)	MTS	-	resection	normal	not SF
cell-cell adhesion, protein translation and modification	25. *PCDH19* c.1123G>T (p.Asp375Tyr)	negative	-	-	-	-
	26. *PKNP* c.1253_1269dup (p.Thr424fs)	microcephaly	-	-	-	-
	27. *QARS* c.2084+2_2084+3del (p.?)	negative	-	-	-	-
neuronal migration, neurogenesis	28. *ARX*[a]	polymicrogyria	-	hemispherotomy	no pathology	SF
other causes	29. Xq25 deletion (122,97-123,02 Mb; ~50 kb)	negative	-	-	-	-
	30. 1q44 deletion[a]	MTS	-	disconnection	no pathology	not SF
	31. 2q23.1-q23.3 deletion (RP11-329H24-RP11-21M18) x1	negative	-	-	-	-
	32. 12p13.33 deletion (23aq2,123-1,380,788)x1 (~1,1Mb)	§	-	resection	MTS	not SF
	33. ringchromosome 20 mosaicism, r(20): 40%[b]	negative	-	-	-	-
	34. ringchromosome 20 mosaicism, r(20): 50%[b]	negative	-	-	-	-

pathway	pathogenic variant (n=40)	MRI	iEEG	surgery	pathology	FU surgery 1y
other causes	35. ringchromosome 20 mosaicism, r(20): 59%[b]	FCD	-	-	-	-
	36. *NF1*[a]	hemiatrophy	-	hemispherotomy	reactive changes	SF
	37. *NF1* 2252-2A>G, exon 14	FCD	-	resection/disconnection	mMCD	not SF
	38. *ANKRD11* (KBG syndrome)[a]	FCD	-	hemispherotomy	normal	SF
	39. *NBEA*c.8401G>A (p.Glu2801Lys)	negative	-	-	-	-
	40. *WDR26*[a]	hemiatrophy	-	hemispherotomy	reactive changes	not SF

[a] exact information on pathogenic variant characteristics could not be retrieved
[b] % observed in proband's lymphocytes

Part II

5

GATOR1-related focal cortical dysplasia in epilepsy surgery patients and their families: a possible gradient in severity?

Barbora Benova, Maurits W.C.B. Sanders, Anna Uhrova-Meszarosova, Anezka Belohlavkova, Barbora Hermanovska, Vilem Novak, David Stanek, Marketa Vlckova, Josef Zamecnik, Eleonora Aronica, Kees P.J. Braun, Bobby P.C. Koeleman, Floor E. Jansen, Pavel Krsek

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ABSTRACT

Objective

Variants of GATOR1 genes represent a recognised cause of focal cortical dysplasia (FCD), the most common structural aetiology in paediatric drug-resistant focal epilepsy. Reports on familial cases of GATOR1-associated FCD are limited, especially with respect to epilepsy surgery outcomes.

Methods

We present phenotypic manifestations of four unrelated patients with drug-resistant focal epilepsy, FCD, and a first-degree relative with epilepsy. All patients underwent targeted gene panel sequencing as part of the presurgical work-up. A literature search was performed to compare our findings with previously published cases.

Results

The children (probands) had a more severe phenotype than their parents, including drug-resistant epilepsy and developmental delay, and did not achieve postsurgical seizure-freedom. All patients had histopathologically confirmed FCD (types IIa, IIb, Ia). In patient 1 and her affected father, we detected a known pathogenic *NPRL2* variant. In patients 2 and 3 and their affected parents, we found novel likely pathogenic germline *DEPDC5* variants. In family 4, we detected a novel variant in *NPRL3*. We identified 15 additional cases who underwent epilepsy surgery for GATOR1-associated FCD with a positive family history of epilepsy in the literature; in 8 of 13 cases with parental testing, the variant was inherited from an asymptomatic parent.

Conclusion

The presented cases displayed a severity gradient in phenotype with children more severely affected than the parents. Although patients with GATOR1-associated FCD are considered good surgical candidates, postsurgical seizure outcome was poor in our familial cases, suggesting that accurate delineation of the epileptogenic zone may be more challenging in this subgroup of patients.

Introduction

Focal cortical dysplasia (FCD) is the most common aetiology of drug-resistant structural epilepsy in children and young adults undergoing epilepsy surgery,[1,2] and 62–72% of patients achieve seizure-freedom after surgery.[2,3] FCD has long been considered a sporadic condition despite evidence of rare familial cases, initially without the identification of genes involved.[4] Scheffer et al. identified germline pathogenic variants in *DEPDC5* in families with multiple members affected with FCD, focal epilepsy and psychiatric comorbidities.[5] Later reports showed that *NPRL2* and *NPRL3*, two other components of the GATOR1 complex, are also involved in the pathogenesis of FCD and focal epilepsy.[6–8] Other studies provided evidence that FCD can occur as a result of somatic or somatic second-hit mutations in *MTOR*, *TSC1* and *TSC2*,[9,10] *DEPDC5*,[11] other genes of mTOR signalling pathway, and *SLC35A2*.[12,13]

To date, there are no reports on the effects of specific genetic variants on the outcomes of epilepsy surgery in patients with FCD. A recent review of 24 studies comprising 82 patients who underwent surgery and had a genetic cause of refractory epilepsy showed that the seizure outcome differed between pathogenic variants; patients with germline pathogenic variants in genes coding for ion channels and synaptic proteins achieved seizure-freedom significantly less often (14%) than patients with germline or somatic pathogenic variants in genes of the mTOR pathway (58% and 83% respectively).[14]

In many centres, genetic testing has not yet been routinely included as a part of the presurgical evaluation despite increasing evidence for its value in epilepsy surgery decision making.[15] Here, we present case reports of four probands selected from the databases of epilepsy surgery programmes at the respective centres for paediatric epilepsy surgery who underwent resective epilepsy surgery for drug-resistant epilepsy, had a positive family history of epilepsy, and in whom targeted gene panel sequencing of genes associated with malformations of cortical development (MCD) revealed a germline variant in GATOR1 genes.[1–3] Next, we summarize findings from previous studies that reported patients with germline variants in GATOR1 and familial FCD and epilepsy who underwent epilepsy surgery to delineate key features of the phenotype, along with postsurgical outcome.

Methods

Patient selection and clinical investigation

The probands were selected from the paediatric epilepsy surgery programme databases of Motol University Hospital (MUH), Prague, Czech Republic or the University Medical Centre (UMC) Utrecht, the Netherlands (search was performed in May 2020), when they met the following inclusion criteria: results of epilepsy surgery for drug-resistant epilepsy available, positive family history of epilepsy, a confirmed germline pathogenic or likely pathogenic variant in either of the genes of GATOR1 complex (*DEPDC5, NPRL2, NPRL3*), and histopathologically confirmed FCD. The patients or their legal representatives provided informed consent for genetic testing and for participation in the study. The probands underwent standard presurgical evaluation, including brain MRI (1.5T or 3T, with dedicated epilepsy protocols), video-EEG, neuropsychological examination, and functional neuroimaging (functional MRI, positron emission tomography [PET], single-photon emission computed tomography [SPECT], and magnetoencephalography [MEG] when indicated). Initially, all patients underwent resective surgery; one patient was eventually treated with a vagus nerve stimulator after two unsuccessful resective surgeries. Resected brain tissue samples were histopathologically evaluated and classified in accordance with the ILAE guidelines

by experienced neuropathologists (EA, JZ).[16,17] All families were provided with genetic counselling by a medical geneticist.

Genetic studies

For germline genetic testing, we used SureSelectXT (Agilent, Santa Clara, CA, USA) system for targeted enrichment of coding regions of selected genes associated with MCD or focal epilepsy (for the complete list of genes, see **supplementary materials**). Raw sequencing data were processed by standard bioinformatics tools for sequence alignment (assembly GRCh37/hg19) and variant identification, including SureCall and NextGene software tools and Genome Analysis Toolkit (GATK)-based analysis pipeline (Broad-Institute, USA). We filtered variants with a minor allele frequency of <1% in population-based databases, including gnomAD (Broad Institute, USA) and 1000 Genomes (IGSR, UK) and compared them to disease databases (HGMD Professional, UK). To validate the variants identified by NGS, we performed Sanger sequencing in the probands and in the parents for segregation analysis. In patients 2 and 4, targeted gene panel sequencing of the affected dysplastic brain tissue sample was performed to detect somatic variants, as previously published.[12] Variants were classified according to ACMG guidelines.[18]

Literature search

We compared clinical and genetic features of our patients, including information on epilepsy surgery outcome, with previously published reports of patients with GATOR1-associated FCD and a family history of epilepsy who underwent epilepsy surgery (**table 1** and **table 2**). The literature search was limited to publications on GATOR1 complex genes (*DEPDC5*, *NPRL2*, *NPRL3*) that explicitly reported a histopathological diagnosis of FCD in patients with at least one family member affected by epilepsy (search date March 2020; searched terms: GATOR1, *NPRL2*, *NPRL3*, *DEPDC5*, focal cortical dysplasia).

Results

We selected four unrelated two- and three-generational families with probands who fulfilled the inclusion criteria. All families were provided with genetic counselling by a medical geneticist. Postsurgically, the patients were followed up by local paediatric neurologists and at the outpatient clinic of Motol Epilepsy Centre and UMC Utrecht (PK, FEJ).

Family 1

1.1 Clinical and epilepsy phenotype

The proband was born to a healthy mother after an uneventful pregnancy with no perinatal risks. At 11 months of age, she first manifested focal tonic and hypermotor seizures with a left-sided predominance. The seizure semiology remained consistent, with daily frequency, occurring predominantly at night. On EEG, frequent epileptiform discharges over the right frontal lobe were detected; the MRI showed features of FCD spanning over the entire right frontal lobe (**figure 1a**). Before the onset of epilepsy, she had normal neurodevelopment; with ongoing seizures, her developmental pace decreased, and later she began to manifest first signs of attention deficit and hyperactivity. Within one year, she tried five antiepileptic drugs (AED), without lasting effect on seizure frequency and she was referred for presurgical evaluation.

1.2 Epilepsy surgery outcomes

At the age of 3 years, the patient underwent one-stage ECoG-guided surgical resection of the right frontal lobe with sparing of the central area. Histopathological examination of the resected tissue confirmed the diagnosis of FCD type IIa (**figure 2a**) with no features of FCD in the surgical margins. The patient became seizure-free and development began to accelerate significantly. After seven months, her seizures relapsed, unresponsive to AED adjustments. Seizure semiology changed and she manifested paroxysmal awakenings from sleep, behavioural arrest with inconstant oroalimentary automatisms and vegetative features. The MRI showed an area of suspected residual FCD in the dorsal orbitofrontal area and right temporal pole, confirmed by signs of ictal hyperperfusion on SPECT. Video-EEG showed ictal activity and interictal epileptiform discharges in the right temporal area; these were not seen on previous video-EEG recordings. The patient underwent second resective epilepsy surgery of the remaining orbitofrontal cortex and a part of the right temporal lobe at the age of 4.5 years that led only to temporary seizure relief. Histopathological assessment showed FCD IIa. Due to ongoing seizures, she underwent S-EEG which was inconclusive, thereby preventing further surgical treatment. A vagus nerve stimulator was implanted. Ongoing seizures were presumed to originate from incompletely removed epileptogenic network comprising a potential residual FCD. Currently, she is 14 years old, and experiences weekly nocturnal seizures, despite the combination of three AED, and she has mild to moderate intellectual disability.

1.3 Family history and genetic findings

The proband's father had normal development but has had focal epilepsy since 12 years of age with no clear evidence of FCD on a standard MRI (**table 1**). His seizures are currently controlled with AED. The proband's sister never experienced seizures and has normal cognitive skills, as well as the father's parents and his sister. Targeted gene panel sequencing revealed a previously published pathogenic germline variant NPRL2:NM_006545.4:c.100C > T(p.Arg34*) in the patient (variant allele frequency [VAF] of 44%) and the father (VAF of 46%; based on BAM file from the NGS data), absent from either GnomAD or 1000 Genomes databases, present in HGMD database.[7,19] Genetic testing in the patient's sister is ongoing. Somatic gene mutation testing of the FCD brain tissue sample was not performed.

Family 2

2.1 Clinical and epilepsy phenotype

The proband was born in an otherwise uncomplicated labour to a previously healthy mother with a history of gestational diabetes. He manifested focal seizures at two weeks evolving to infantile spasms at 2 months of age. His first EEG showed frequent multifocal epileptiform discharges and hypsarrhythmia, with seizures originating from right parietal and occipital areas. MRI showed a T2 hypointense lesion in the temporo-occipital border of the right hemisphere (**figure 1b**), consistent with FCD [20], and concordant with the ictal onset zone. Neurodevelopment progressed adequately in social and mental skills but with delay in gross and fine motor skills. Initial treatment with vigabatrin led to limited seizure control; therefore, the patient was started on adrenocorticotrophic hormone (ACTH). Despite multiple AEDs, the patient's seizures continued, and he was referred for presurgical evaluation.

Table 1. Summary of clinical findings (extends over both pages of this spread).

	sex	gene	variant (cDNA)	variant (pDNA)	RefSeq	ACMG class	segregation analysis	epilepsy phenotype	age at sz onset
patient 1	F	*NPRL2*	NM_006545.4:c.100C>T	p.Arg34*	NM_006545.4	pathogenic	inherited (affected father)	FE	11 m
parent 1	M	*NPRL2*	NM_006545.4:c.100C>T	p.Arg34*	NM_006545.4	pathogenic	affected	FE and GTCS	12 y
patient 2	M	*DEPDC5*	NM_001242896.1:c.2294del	p.Gly765Alafs*29	NM_001242896.1	likely pathogenic	inherited (affected father)	IS, FE	2 m
parent 2	M	*DEPDC5*	NM_001242896.1:c.2294del	p.Gly765Alafs*29	NM_001242896.1	likely pathogenic	affected	FE, FBTCS	19 y
patient 3	M	*DEPDC5*	NM_001242896.1:c.2843T>C	p.Leu948Pro	NM_001242896.1	likely pathogenic	inherited (affected mother)	FE, EEG status epilepticus	3 d
parent 3	F	*DEPDC5*	NM_001242896.1:c.2843T>C	p.Leu948Pro	NM_001242896.1	likely pathogenic	affected	GTCS	10 y
patient 4	F	*NPRL3*	NM_001243248.1:c.[898_900del]	p.Ile300del	NG_029669.1 g.[50423_50425del]	pathogenic	inherited (affected mother)	FE	0 m
parent 4	F	*NPRL3*	NM_001243248.1:c.[898_900del]	p.Ile300del	NG_029669.1 g.[50423_50425del]	pathogenic	affected sister	FE	13 y

	pre/perinatal complications	MRI findings	cognitive functions	psychiatric comorbidity	epilepsy surgery	histopatho-logical diagnosis	epilepsy surgery outcome	length of FU	seizure-free	last known AED	originally described	notes
	none	FCD – R frontal lobe	mild to moderate id	no	2 resective surgeries, 1 seeg, 1 vns implantation	FCD IIa	Engel III	9 y	no	LCS, CLB, PGB	Ricos et al.	
	n/a	Normal	normal	n/a	none	N/A	N/A	N/A	yes	VPA, LEV		
	gestational diabetes	FCD – R temporo-occipital	motor skill delay	no	1 resective surgery	FCD IIa	Engel IV	2 y	no	VGB, CBZ, VPA, LEV	previously unreported	
	n/a	Normal	normal	n/a	none	N/A	N/A	N/A	no	None		
	thyroid substitution	FCD – R frontal lobe	profound id, asd	aggression and autoaggression	1 resective surgery	FCD IIb	Engel IV	14 y	no	VGB, PHT, CLB, PRM, PP	previously unreported	
	n/a	Normal	normal	n/a	none	N/A	N/A	N/A	yes	PHT		
	none	FCD – L frontal lobe	moderate id, impaired motor development	none	1 resective surgery (25-1-2019)	FCD IIa (IIb not excluded)	Engel ID	1 y 3 m	yes	LEV, VGB	previously unreported	
	none	Normal	normal	none	none	None	N/A	N/A	yes	OXC		sporadic seizures

Table 2. Summary of literature findings (extends over both pages of this spread).

reference	gene	variant (cDNA)	variant (pDNA)	segregation analysis	affected family member	epilepsy phenotype	age at onset
Baldassari et al., 2019	*DEPDC5*	c.279+1G>A	p.?	inherited (affected mother)	mother	SHE	6 years
	DEPDC5	c.1264C>T	p.Arg422*	inherited (asymptomatic father)	member of paternal branch	SHE	5 months
	DEPDC5	c.1310delA	p.Asn437Metfs*21	inherited (asymptomatic mother)	unknown	FE	2 months
	NPRL2	c.100C>T	p.Arg34*	inherited (affected father)	father	SHE	11 months
	NPRL3	Deletion (exons 5–10)	p.?	present in affected offspring	maternal branch, 3 affected	FE	3 years
Scerri et al., 2015	*DEPDC5*	c.1663C>T	p.Arg555*	inherited (affected father)	father	FE	neonatal
Leventer et al., 2014	*DEPDC5*	c.1663C>T	p.Arg555*	inherited (affected father)	father	FE	neonatal
Baulac et al., 2015	*DEPDC5*	c.715C>T	p.Arg239*	inherited (asymptomatic mother)	multiple family members	FE	neonatal
	DEPDC5	c.715C>T	p.Arg239*	inherited (asymptomatic father)	multiple family members	FE	2 years
	DEPDC5	c.484-1G>A	p.?	inherited (asymptomatic father)	sister	FE	18 months
	DEPDC5	c.1759C>T	p.Arg587*	present in affected uncle	uncle (parents not tested)	FE	4 years
	DEPDC5	c.1759C>T	p.Arg587*	present in affected nephew	nephew (parents not tested)	FE	4 years
Sim et al., 2016	*NPRL3*	c.1375_1376dupAC	p.S460Pfs*20	inherited (asymptomatic mother)	brother	FE	neonatal
	NPRL3	c.1375_1376dupAC	p.S460Pfs*20	inherited (asymptomatic father)	brother	FE	7 years
Weckhuysen et al., 2016	*NPRL2*	c.68_69delCT	p.Ile23Asnfs*6	inherited (asymptomatic father)	multiple family members	FLE	3 years
	NPRL3	c.1270C>T	p.Arg424*	inherited (affected mother)	mother	FLE	2 months

	epilepsy phenotype (affected family member)	MRI findings	cognitive functions	psychiatric comorbidity	histopathological diagnosis	epilepsy surgery outcome	length of FU	notes
	N/A	FCD	normal	no	FCD IIa	Engel I	15 months	–
	FE, undetermined	FCD	psychomotor (language) impairment	no	FCD IIa	Engel I	5.3 years	–
	N/A	FCD	mild ID	ASD	FCD Ic	N/A	–	–
	N/A	FCD	mild to moderate ID	no	FCD IIa	Engel III, VNS	9.3 years	from 2nd surgery
	FLE	normal	normal	no	FCD I	drug-resistant	8 years	–
	FE, undetermined	hemispheric FCD	N/A	N/A	FCD IIa	Engel I	–	2 brothers, 2 publications of the same family
	FE, undetermined	quadrantic FCD	N/A	N/A	FCD IIa	Engel I	–	2 brothers, 2 publications of the same family
	FE, FCD on MRI	FCD	normal	depression, PNES	fragmented specimen, inconclusive	Engel IV	N/A	–
	FE, FCD on MRI	normal	normal	No	FCD I	Engel I	13 years	
	N/A	normal	normal	depression, transitory psychosis	FCD IIa	Engel I	4 years	–
	FE	FCD	normal	no	FCD IIa	Engel I	7 years	–
	FE	FCD	normal	impulsivity	fragmented specimen, inconclusive	Engel III (' worthwile improvement')	N/A	–
	N/A	quadrantic FCD	global developmental delay	N/A	FCD IIa	Engel II	3 years	–
	N/A	FCD	normal	N/A	FCD IIa	Engel I	1 years	–
	FE, undetermined	normal	N/A	N/A	FCD Ia	Engel II	9 years	–
	FLE	FCD, hippocampal atrophy	N/A	N/A	FCD IIa, hippocampal sclerosis	Engel II	9 years	–

2.2 Epilepsy surgery outcome

The patient underwent one-stage ECoG-guided tailored resection of the right temporo-parietal cortex at 1 year of age that, however, led only to temporary seizure relief. Histopathological confirmation of FCD IIa was obtained. Three months after surgery he began to manifest sleep-related focal seizures with paroxysmal awakening and inconstant twitching of the right arm. Repeated video-EEG showed ongoing epileptiform activity over the right occipital, temporal and parietal regions with rapid spread of ictal activity over the entire right hemisphere. On repeated MRI, at age 2 years, we detected a more extensive dysplastic area that encompassed the occipital and part of the temporal lobe. Developmentally, his deficit in gross motor skills persisted, along with newly-appearing deficits in fine motor skills. The patient underwent a second surgery at the age of three years consisting of an ECoG-guided multilobar surgical resection of the remaining right occipital lobe, temporal lobe and a part of parietal lobe. Histopathological examination showed clusters and vertical columns of neurons, along with immature and giant neurons; however, without the dysmorphic neurons typical for FCD IIa, suggestive of a lesion gradient.

2.3 Family history and genetic findings

The patient's father had normal development and was diagnosed with epilepsy at 19 years of age, initially presenting with a tonic-clonic seizure of unknown origin. With limited compliance to medication, he experiences stereotyped focal seizures sometimes evolving to bilateral tonic-clonic seizures. He had no MRI performed. The mother had normal development, never experienced seizures and her family history is negative for epilepsy or other neurological conditions. The patient's sibling, as well as the father's parents (the patient's grandparents) have no history of seizures or developmental disorders. Father's grandmother (the patient's great-grandmother) had seizures of unknown origin, possibly related to alcohol abuse. Targeted gene panel sequencing revealed a novel likely pathogenic germline variant DEPDC5:NM_001242896.1:c.2294del(p.Gly765Alafs*29), present in the patient (VAF of 44%) and in the affected father (heterozygous signal from Sanger sequencing), absent from either GnomAD or 1000g databases. The mutation leads to a frameshift at the position of codon Gly765 and to the insertion of a stop codon at position 29 of the new reading frame. The variant segregates with the condition. Therefore, we classify it as likely pathogenic. No second-hit somatic variant was detected in the brain tissue.

Family 3

3.1 Clinical and epilepsy phenotype

The patient was born at term to a mother treated with phenytoin for focal epilepsy, with thyroid hormone and folic acid substitution during pregnancy. On the third day of life, he manifested left-sided focal tonic-clonic seizures with nystagmus. Later, he also experienced head drops. His MRI showed extensive cortical dysplasia of the right frontal lobe and central sulcus (**figure 1c**). On the initial EEG, he displayed a 'burst-suppression' pattern and later continuous epileptiform discharges over the right frontal and temporal regions. Since neonatal age, his neurological findings were abnormal, including hypotonia and signs of left-sided hemiparesis. His neurodevelopment was delayed from birth and regressed significantly during periods of seizure decompensation. Despite a combination of multiple AEDs, he never experienced a sustained seizure-free period; and was therefore referred for evaluation for epilepsy surgery.

3.2 Epilepsy surgery outcome

At 2.5 years of age, he underwent ECoG-guided resection of the right frontal lobe with sparing of precentral primary motor cortex; at this age, S-EEG was impossible to perform due to insufficiently ossified skull bones. Histopathological examination confirmed the diagnosis of a complex malformation of cortical development combining features of FCD type IIb (**figure 2c**) with areas of polymicrogyria and pachygyria. Postsurgically, the patient was seizure-free for 1.5 years. His developmental skills progressed but only slowly and he developed autism spectrum disorder with major impairment of behaviour. His seizures relapsed, probably due to residual dysplastic tissue seen on the postsurgical MRI in the originally spared primary motor cortex. The parents did not wish to proceed with a second surgery. In further course, he required a combination of three to five AED. He died at 17 years of pneumonia and septic shock. The autopsy report did not reveal residual FCD tissue in the proximity of the original cortical resection. However, sampling of post-mortem tissue may have been incomplete. Alternatively, glial scarring in the resection margins may have prevented the histopathological recognition of residual FCD tissue.

3.3 Family history and genetic findings

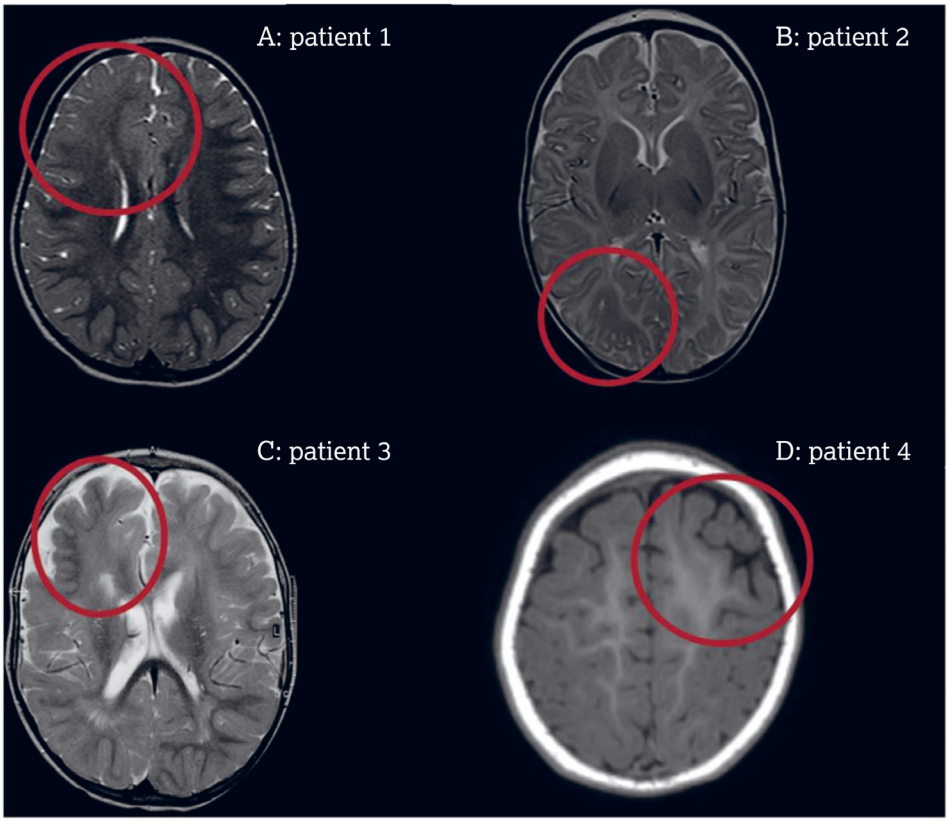
The patient's mother with normal neurological functioning manifested a tonic-clonic seizure at 10 years of age. Later, her seizures were preceded by auras followed by versive turning to the left side. Initially the seizures occurred rarely (once per year), and currently she has been seizure-free for many years on a low daily dose of phenytoin. No other family members had epilepsy. We detected a previously unreported probably pathogenic germline variant in DEPDC5:NM_001242896.1:c.2843T > C(p.Leu948Pro) absent from either GnomAD or 1000 Genomes databases in the patient (VAF of 49%) and in the affected mother in a heterozygous state. The mutation occurred in a highly-conserved nucleotide (PhyloP 4.73) and a highly conserved amino acid. The in-silico predictions classified the variant as deleterious (SIFT) and disease-causing (MutationTaster) and the variant segregates with the condition. Therefore, we classify it as probably pathogenic. Somatic mutation testing on resected tissue was not performed.

Family 4

4.1 Clinical and epilepsy phenotype

The proband was born at term to a mother with focal epilepsy, with long-term seizure-freedom on AED treatment. The patient developed frequent neonatal seizures with rhythmic myoclonic jerks of the right hand and foot, eye blinking and chewing movements. MRI of the
brain showed an area with a loss of grey-white matter differentiation suggestive of FCD in the left frontal area adjacent to the central sulcus (**figure 1d**). Her EEG confirmed ictal onset in the left central area. Neurological examination revealed a mild right-sided hemiparesis. Neuropsychological examination at 6 months of age showed significant developmental delay.

Figure 1. MRI findings; 1a–1d. Patients 1–4, asterisks denoting FCD.



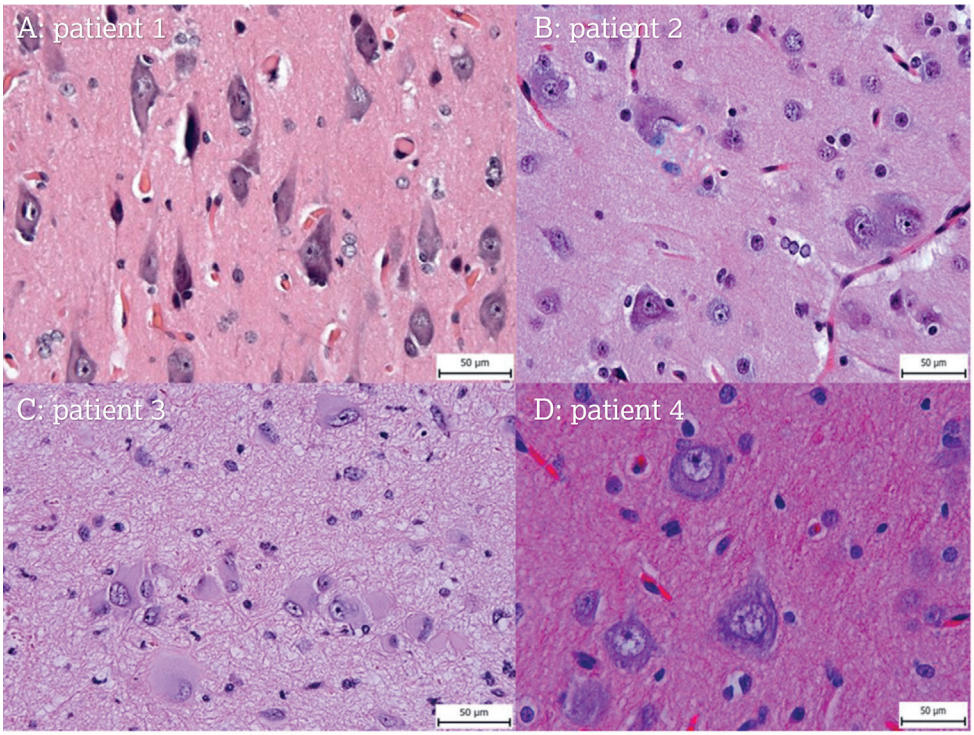
4.2 Epilepsy surgery outcome

At 11 months of age, she underwent an ECoG-guided tailored resection of the left frontal FCD lesion; because the lesion was clear and well-demarcated, S-EEG was not indicated. Histopathological evaluation revealed FCD IIa. She experienced no seizures in the first few months after surgery and follow-up EEG showed no epileptiform activity. However, her seizures recurred after the AEDs were tapered down and discontinued. At 14 months of follow-up, she has been seizure-free on the combination of two (reintroduced) AEDs (LEV, VGB). Her development is progressing but still severely delayed.

4.3 Family history and genetic findings

The proband's mother and the mother's sister were diagnosed with focal epilepsy during childhood and were successfully treated with AEDs. The mother's parents never experienced seizures. We detected a pathogenic NPRL3:NM_001243248.1):c.[898_900del] germline variant in the patient and in the affected mother, absent from either GnomAD or 1000 Genomes databases. Somatic gene mutation testing revealed no causal pathogenic variant.

Figure 2. Histopathological findings; 1a–1d. Patients 1-4. Hematoxylin-eosin staining.



Previously published findings on GATOR1-related focal cortical dysplasia

We found six studies that altogether reported sixteen patients who fulfilled the same inclusion criteria as those in our cohort (including patient 1 from our series, previously reported in the study by Baldassari, et al.). In 13 of the 16 cases, the parents underwent genetic testing for GATOR1 variants; in 8 of the 13 cases the variant was inherited from an asymptomatic parent, whereas in the remaining 5 of 13, the parents were also affected. In 3 of the 16 cases, the variant was present in another affected family member. Eight of the 16 patients achieved complete seizure-freedom (Engel class I): in 4 of these 8, the variant was inherited from an asymptomatic parent, in 3 of 8, the parents were also affected; and in 1 of 8, the parents were not tested. The majority of patients (10 of 16) had histopathologically confirmed FCD type IIa; in the remaining cases, the authors reported either FCD type I (4 of 16) or inconclusive findings (2 of 16). All cases were classified according to the ILAE guidelines.[17] All patients underwent high-resolution MRI (1.5T or 3T) as a part of standard presurgical evaluation.

Discussion

Here, we report four familial cases of GATOR1-associated FCD and focal epilepsy; the probands underwent resective epilepsy surgery with histopathological confirmation of FCD. Our cases carry GATOR1 complex variants, three of which have not been previously reported, and demonstrate two notable features: increasing phenotypic severity across generations in all four families, and relatively poor surgical outcomes. All patients underwent epilepsy surgery after a thorough presurgical evaluation following internationally-approved guidelines and local practices.[21] Currently, the role of molecular genetic findings in presurgical evaluation is under investigation. None of the patients achieved sustained seizure freedom without ongoing antiseizure medication. This might have resulted from incomplete surgical resection, from the underlying genetic aetiology, or the combination thereof. Generally, patients with FCD type II are considered good surgical candidates who achieve postsurgical seizure-freedom in 62% of cases,[2] and in a more recent study even 67% at five years after surgery.[22] Outcomes specific for GATOR1-positive patients have only been published on a small surgical subgroup where 6/10 patients were seizure-free one year after surgery.[23] We found two novel variants in *DEPDC5* (NM_001242896.1:c.2294del, p.Gly765Alafs*29 and NM_001242896.1:c.2843T > C p.Leu948Pro) and a novel variant in *NPRL3*: NM_001243248.1:c.[898_900del]; both genes with autosomal dominant inheritance associated with focal epilepsy and FCD. In contrast, the variant *NPRL2*: NM_006545.4:c.100C > T (p.Arg34*), also a gene with autosomal dominant inheritance, was initially described in patients with sleep-related hypermotor epilepsy without evidence of FCD; our patient has an MRI- and histopathologically confirmed FCD in addition to sleep-related seizures whereas her affected father has no MRI features of FCD and is currently seizure-free. Other reported variants in *NPRL2* (p.Thr110Ser and p.Asp214His), however, were detected in patients with brain lesions; the variant p.Thr110Ser was present also in an affected individual.[7]

The most notable clinical feature observed in our GATOR1 cases was a consistently more severe presentation in the probands than in the affected parents. None of the parents required epilepsy surgery and therefore we had no histopathological confirmation of FCD. Parents' MRI scans, when available, showed no signs of FCD but were performed many years ago without the use of dedicated epilepsy protocols. None of the parents displayed signs of intellectual disability, in contrast to varying levels of developmental delay in the four probands. Three of the four affected variant-positive parents were seizure-free; in contrast to three of four probands with postoperative persistent seizures. Based on the observations in our series, we hypothesised that the more severe phenotypic presentation in the second generation of affected family members in our cohort might be a common feature of familial GATOR1-related FCD. In 8 of 13 previously published cases with parental testing, the pathogenic variant was inherited from an asymptomatic parent, suggesting a possible phenotypic gradient from a pathogenic variant-positive parent to offspring. On the other hand, not all studies corroborate these observations. For instance, in the series of Baldassari, et al., the proband had drug-resistant epilepsy caused by FCD type I and underwent two unsuccessful epilepsy surgeries, whereas her child developed seizures at a later age that were adequately controlled with AED.[23]

Multiple mechanisms may explain this phenotypic variability, including incomplete penetrance and reproductive disadvantage, as severely affected patients rarely have offspring; therefore, phenotpyic variability may not become evident within these families. Another possible explanation why GATOR1-positive FCD patients manifest a more severe phenotype than those without FCD might be the presence of somatic second-hit variant in the dysplastic brain tissue of patients with FCD. Even though the second-hit hypothesis has been confirmed as the pathogenic mechanism of

FCD formation in some patients with *DEPDC5* variants [11], in our cohort we were unable to detect a second-hit variant in the dysplastic brain tissue of patients 2 and 4. Although the variant might be present at a low variant allele frequency, below the detection level of the method, other factors might still play a role. The proportion of variant-positive FCD patients with two-hit GATOR1 variants ranges from 0.9% to 3.2% as reported in the largest surgical series with genetic testing yet published.[12,13]

Ultra-deep sequencing required for variant detection in FCD tissue samples still represents a technical challenge for routine diagnostics, which might explain the low number of patients currently tested for the presence of second-hit variants in the brain tissue, and the low reported proportion of detected somatic variants. Despite the technical obstacles and the still limited diagnostic yield, molecular characterisation of brain tissue samples obtained in epilepsy surgery has gained interest in the neuropathological diagnostic process and might become a cornerstone of diagnosis and prognosis in the near future,[23] as is the case in the molecular characterisation of brain tumours.[24]

Apart from the incomplete penetrance and the presence of second-hit variants in FCD tissue samples, low-level mosaicism might be another explanation for the phenotypic variability among GATOR1-associated FCD patients and the gradient between parents and offspring. Baulac et al. reported in their first publication of DEPDC5-associated familial focal epilepsy and FCD a family in which the asymptomatic father carried a pathogenic variant with only a 5% level of mosaicism.[25] He transmitted the variant to the offspring, one of whom had FCD and drug-resistant focal epilepsy; variant allele frequency (VAF) in the offspring was not noted. In our cohort, the affected probands carried the pathogenic variant with a VAF of 44%, 44% and 49% (patients 1–3), respectively. The father of patient 1 carried the variant with a VAF of 46% (based on BAM file from the NGS data); the other two parents underwent Sanger sequencing only and therefore we could not ascertain the precise VAF. Given the detection sensitivity (approximately 15%) and the heterozygous signal observed on Sanger sequencing, we estimate the VAF in these two parents to be approximately 50%. Our results therefore seem to contradict the hypothesis that the severity of phenotype depends on the VAF either in the parent or in the affected offspring. However, we report only a small number of cases, and larger studies on phenotypic-VAF correlations in patients with GATOR1 pathogenic variants are lacking in the literature.

In contrast, the role of parental mosaicism has been established in patients with seemingly 'de novo' epileptic encephalopathies caused by pathogenic variants in genes with autosomal dominant inheritance, including patients with SCN1A-associated Dravet syndrome.[27–29] The authors found that 58% of parents of children with seemingly 'de novo' variants were in fact mosaic carriers with VAF ranging from less than 1% to 30.6%.[26-28] If the parent was mildly affected, the chances that mosaicism was detected in the parents increased.[27] To summarize, our results and the published GATOR1 cases do not unequivocally suggest that phenotypic gradient is caused by parental mosaicism; however, the cohort size prevents firm conclusions. Future studies involving more GATOR1 families with deep sequencing of affected and unaffected family members might clarify the role of mosaicism in the severity of symptoms in these patients.

Our study contributes to the limited literature on familial FCD and focal epilepsy; we identified only 16 previously reported individuals with GATOR1 pathogenic variants who underwent epilepsy surgery with a confirmed diagnosis of FCD and a family history of epilepsy (**table 2**).[6,8,19,25,29] Our findings highlight phenotypic variability and a possible phenotypic gradient in GATOR1-associated FCD. However, future studies are needed to clarify the role of incomplete penetrance, second-hit variants and parental mosaicism in the phenotypic presentation of GATOR1 patients and their impact on surgical outcome.

The limitations of our study include the small number of cases, a possible selection bias and the lack of MRI in 2 of 4 parents. We could not obtain MRI from all the parents because MRI was either unavailable during their childhood and adolescence or because they are currently seizure-free and MRI was not clinically indicated.

This report highlights the importance of genetic testing in epilepsy surgery programmes. Our data, together with previously published reports, suggest that patients with GATOR1-related FCD and a positive family history of epilepsy may represent particularly challenging candidates for epilepsy surgery, with some requiring invasive S-EEG studies and repeated surgeries to achieve freedom from seizures. The majority of our patients were too young to undergo S-EEG studies at the time of their surgeries; in our centres, the youngest possible age for S-EEG is three years. The main issues with S-EEG in small children include unstable skull due to incomplete ossification and insufficient cooperation. Otherwise, S-EEG remains an important diagnostic tool to delineate precisely the margins of the epileptogenic zone in selected epilepsy surgery candidates, regardless their genetic aetiology. However, there is no defitinive evidence that tailoring resection using S-EEG improves seizure outcome. In addition to intractable seizures, these patients were reported to have psychiatric comorbidities and intellectual disability to varying degrees. We therefore strongly advocate timely referral of patients with drug-resistant epilepsy and a positive family history to tertiary epilepsy centres for presurgical evaluation. Future research should focus on the identification of clinical, neuropathological and molecular-genetic factors associated with surgical outcome in this challenging group of patients.

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6

Somatic variant analysis of resected brain tissue in epilepsy surgery patients

Maurits W.C.B. Sanders, Bobby P.C. Koeleman, Eva H. Brilstra, Floor E. Jansen, Sara Baldassari, Mathilde Chipaux, Nam Suk Sim, Ara Ko, Hoon-Chul Kang, Ingmar Blümcke, Dennis Lal, Stéphanie Baulac, Jeong Ho Lee, Eleonora Aronica*, Kees P.J. Braun*

**These authors jointly directed this work

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ABSTRACT

We studied the distribution of germline and somatic variants in epilepsy surgery patients with (suspected) malformations of cortical development (MCD), who underwent surgery between 2015 and 2020 at the University Medical Center Utrecht (the Netherlands), and pooled our data with four previously published cohort studies. Tissue analysis yielded a pathogenic variant in 203 of 663 (31%) combined cases. In 126 of 379 (33%) focal cortical dysplasia (FCD) type II cases and 23 of 37 (62%) hemimegalencephaly cases, a pathogenic variant was identified, mostly involving the mTOR signaling pathway. Pathogenic variants in 10 focal epilepsy genes were found in 48 of 178 (27%) FCDI/mMCD/mMCD with oligodendroglial hyperplasia and epilepsy cases; 36 of these (75%) were *SLC35A2* variants. Six of 69 (9%) patients without a histopathological lesion had a pathogenic variant in *SLC35A2* (*n*=5) or *DEPDC5* (*n*=1). A germline variant in blood DNA was confirmed in all cases with a pathogenic variant in tissue, with a variant allele frequency (VAF) of ~50%. In seven of 114 patients (6%) with a somatic variant in tissue, mosaicism in blood was detected. More than half of pathogenic somatic variants had a VAF < 5%. Further analysis of the correlation between genetic variants and surgical outcomes will improve patient counseling and may guide postoperative treatment decisions.

Introduction

Epilepsy surgery is the most effective treatment in epilepsy patients who develop refractory seizures, provided that the epileptogenic zone is focal, well delineated, preferably of presumed structural origin, and located outside eloquent areas.[1] Genetic malformations of cortical development (MCD), such as mild MCD (mMCD), mMCD with oligodendroglial hyperplasia and epilepsy (MOGHE), focal cortical dysplasia (FCD; especially FCDIIa and FCDIIb subtypes), and hemimegalencephaly (HMEG) account for more than one-third of children who undergo surgery.[2,3]

Most MCDs are found to arise from variants in genes that encode proteins essential for neurodevelopment. These variants can be detected, de novo or inherited, in the patient's germline or arise during embryogenesis (postzygotic), and lead to somatic mosaicism with variant allele frequencies (VAFs) under 50%. In recent years, genetic testing has been increasingly applied in focal epilepsy patients who are considered for epilepsy surgery, and the outcome may guide the selection of eligible patients for invasive intracranial monitoring and resective surgery.[3–5] Possibilities to comprehensively test epilepsy patients for a genetic etiology with next-generation sequencing techniques (NGS) have expanded and have shown that somatic variants in mTOR pathway genes (*AKT3*, *DEPDC5*, *MTOR*, *PIK3CA*, *RHEB, PTEN, TSC1*, and *TSC2*) or in the UDP-galactose transporter gene *SLC35A2* are the major genetic causes of HMEG, FCD, and pathology-negative epilepsy surgery patients.[6–8] About 70% of reported pathogenic somatic variants have a very low allelic fraction (i.e. VAF < 5%), which is unlikely to be detected by conventional NGS (whole exome sequencing [WES] or whole genome sequencing [WGS]) with low read depth, or by Sanger sequencing of tissue. Nevertheless, somatic variants with a low VAF of merely 1% in the affected brain have been shown to be sufficient to cause refractory epilepsy. This suggests that high-read-depth (deep) sequencing (e.g., ~1000 × read depth), which is mostly cost-effective when performed using a gene panel, is required to determine how variants in MCD genes affect epilepsy.[9]

In the current study, we performed genetic analysis of the resected tissue from patients with (suspected) MCD to identify somatic or germline variants in known focal epilepsy-related genes, with the use of deep sequencing techniques, and pooled the results from our center with the data from four recent cohort studies.[10–13]

Methods

Cohort

Patients who underwent epilepsy surgery between 2015 and 2020 at the University Medical Center Utrecht (the Netherlands [NL]) were enrolled in this study. Patients were eligible if they had an established histopathological diagnosis consistent with mMCD, MOGHE, FCDI or FCDII, or HMEG, or if they were suspected to have an MCD but histopathological examination of resected tissue demonstrated no abnormalities or merely nonspecific reactive gliosis. Neuropathological diagnoses were classified according to the International League Against Epilepsy guidelines.[3] FCDI referred to isolated lesions with cortical dyslamination, which were classified as FCDIa when an excess of microcolumns was observed; FCDII referred to cortical dyslamination and the presence of dysmorphic neurons (DNs), with (FCDIIb) or without (FCDIIa) balloon cells (BCs). mMCD referred to surgical cases with blurred gray-white matter boundaries and excess of heterotopic neurons in cortical layer 1 (mMCD type I) or in the deep white matter (mMCD type II). MOGHE referred to surgical cases with an increase in oligodendroglia and heterotopic neurons in the white matter. DNA for genetic analysis was derived from specimens that were either formalin-fixed and

paraffin-embedded (FFPE) by the neuropathology department or frozen immediately after surgery in liquid nitrogen (fresh frozen [FFZ]). Matched blood DNA samples from the included patients, when available from regular clinical care, were used to investigate the presence of postzygotic mosaicism and germline variants in both brain and peripheral sequencing data. For unmatched samples, variant callers were employed to mitigate the risk of overestimating pathogenicity. All patients consented to participate in this study, as approved by the medical ethics review committee. For the pooled data analysis, we included published data from the other four cohorts according to the methods previously described.[10–13]

DNA extraction from samples & sequencing

Genomic DNA was extracted from FFPE brain samples, using QIAamp DNA FFPE kits (Qiagen). From FFZ brain samples, genomic DNA was extracted using QIAamp DNA Mini kits (Qiagen). For peripheral blood DNA samples, QIAamp DNA Blood Mini kits (Qiagen) were used. Patients were analyzed with the use of two different panels. Panel 1 concerned a hybrid capture-based NGS with a coverage of ~1000 × read depth that required a minimum input of >200 ng DNA, and consisted of the following genes: *BRAF, FGFR1, DEPDC5, NPRL2, NPRL3, AKT1, AKT3, MTOR, PIK3CA, PIK3R2, SLC35A2, TSC1, TSC2, PTEN, RYR3,* and *TBC1D7*. Panel 2 involved amplicon sequencing with a mean coverage of ~1500 × per amplicon, and consisted of the following genes: *BRAF, FGFR1, DEPDC5, NPRL2, NPRL3, AKT1, AKT3, MTOR, PIK3CA, PIK3R2, SLC35A2, TSC1, TSC2, PTEN, TBC1D7, RHEB, PIK3R1,* and *RPS6*. Panel 2 was utilized for cases where only formalin-fixed, paraffin-embedded (FFPE) tissue or tissue with limited DNA content (2–10 ng) was available, and FCDII patients with negative results from panel 1 (after isolating pS6-positive cells with laser capture). For all candidate variants, validation sequencing using targeted amplicon sequencing (MiSeq Dx sequencer; Illumina) was performed. Variants were reported as (likely) pathogenic according to the American College of Medical Genetics and Genomics (ACMG) guidelines adapted for classification of somatic missense variants.14,15 Based on these criteria, we excluded germline variants with uncertain pathogenicity. We classified variants as pathogenic only if they were somatic missense variants in mTOR-pathway activators previously reported, or shown to cause hyperactivation of the mTOR pathway in vitro, or germline/somatic loss-of-function variants in mTOR-pathway repressors or in SLC35A2. Missense variants in TSC1/2 or SLC35A2 were classified as pathogenic only if already reported. Unreported variants were considered likely pathogenic if confirmed as somatic and absent in gnomAD. For PTEN and DEPDC5, single-nucleotide variants resulting in protein truncation or loss of function were considered to be pathogenic because their pathogenic mechanism is loss of function. Germline mutations in TSC1/2 reported as benign or with conflicting interpretation according to the ACMG guidelines were excluded. A full list of genes included in each panel version is available in the supplemental information (supplementary table 2).

Analysis

Differences in proportion of patients with a pathogenic variant between the centers (NL *versus* pooled cohorts) were examined by use of χ^2 tests for categorical variables. Categorical data are presented as percentages. Data analysis was performed with IBM SPSS 23.

Results

Patient characteristics

We included 123 epilepsy surgery patients at our center. In the pooled data analysis (n=663), we further included 80 patients from France,[10] 107 patients from the USA,[11] 170 patients from the Korean study (which reported the results of 177 patients, of whom seven were already included in our cohort),[12] and 180 patients from the USA (Cleveland) and the European Epilepsy Brain Bank (EEBB) consortium study.[13] Matched peripheral DNA samples were available for sequencing in 44 (36%) NL-patients, and 342 of the 663 patients (51%) from all centers combined. Genetic findings of the Dutch cohort and the pooled patient population are listed in **table 1**.

Table 1. Findings (proportional) of genetic testing in brain tissue according to histopathology in the NL-cohort (A; n=123) and in all 4 cohorts combined (B; n=663).

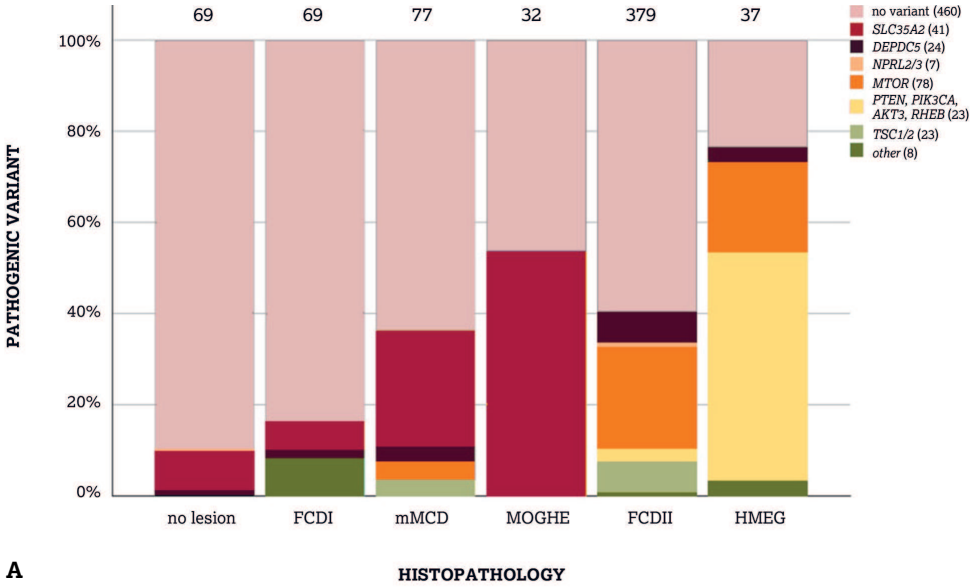
GENETIC+MRI FINDINGS / HISTOPATHOLOGY		N	no pathogenic variant ALL	(likely) pathogenic variant								MRI-negative
				All	SLC35A2	DEPDC5	NPRL2/3	MTOR	PTEN, PIK3CA, AKT3, RHEB	TSC1/2	other	
A: NL	**ALL – no. (%)**	**123**	**84 (68)**	**42 (34)**	**7 (6)**	**7 (6)**	**2 (2)**	**16 (13)**	**3 (2)**	**7 (6)**	**0**	**34 (28)**
	no lesion – no. (%)	26	25 (96)	1 (4)	1 (4)	0	0	0	0	0	0	15 (58)
	FCDI – no. (%)	1	1 (100)	0	0	0	0	0	0	0	0	0
	mMCD – no. (%)	29	19 (66)	10 (34)	5 (17)	1 (3)	0	2 (7)	0	2 (7)	0	7 (25)
	MOGHE – no. (%)	1	0	1 (100)	1 (100)	0	0	0	0	0	0	0
	FCDII – no. (%)	65	33 (51)	32 (49)	0	6 (12)	2 (3)	14 (21)	3 (5)	5 (78	0	11 (17)
	HMEG – no. (%)	1	1 (100)	0	0	0	0	0	0	0	0	0
B: pooled cohorts	**ALL – no. (%)**	**663**	**460 (69)**	**203 (31)**	**41 (6)**	**23 (3)**	**7 (1)**	**78 (12)**	**23 (3)**	**23 (3)**	**8 (1)**	**74 (27)[a]**
	no lesion – no. (%)	69	63 (91)	6 (9)	5 (8)	1 (1)	0	0	0	0	0	52 (76)
	FCDI – no. (%)	69	59 (86)	10 (14)	4 (6)	1 (2)	0	0	0	0	5 (8)	0
	mMCD – no. (%)	77	57 (74)	20 (26)	14 (18)	2 (4)	0	2 (4)	0	2 (4)	0	6 (18)
	MOGHE – no. (%)	32	14 (44)	18 (56)	18 (56)	0	0	0	0	0	0	0
	FCDII – no. (%)	379	253 (67)	126 (33)	0	18 (5)	7 (2)	70 (18)	8 (2)	21 (6)	2 (1)	16 (15)
	HMEG – no. (%)	37	14 (38)	23 (62)	0	1 (3)	0	6 (16)	15 (40)	0	1 (3)	0

[a] no exact information on MRI outcome could be retrieved in 381 patients

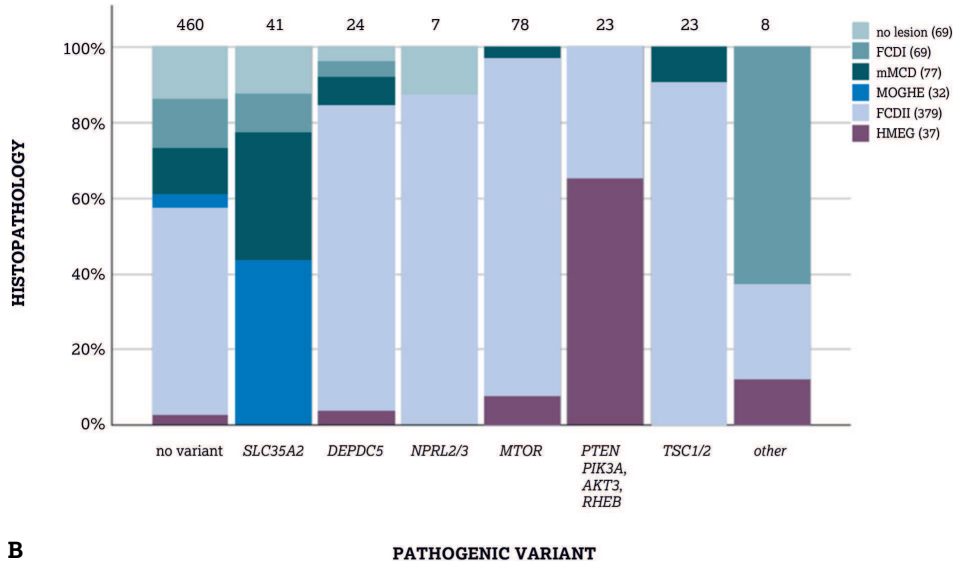
Tissue analysis

Outcomes (pooled) of genetic analysis of brain tissue samples according to histopathological diagnosis are illustrated in **figure 1**.

Figure 1. Distribution (proportional) of outcome of genetic testing (somatic and germline variants) in brain tissue according to histopathology (A) and of histopathology according to outcome of genetic testing in brain tissue (B) in all 4 cohorts combined (n=663).



A



B

There was a similar rate of pathogenic variants (somatic and/or germline) in the NL cohort (42 of 123 cases, 34%) and the pooled cohorts (203 of 663 cases, 31%; p-value of difference = 0.51), comprising pathogenic variants in 10 of 69 FCDI cases (14%), 20 of 77 mMCD cases (26%), 18 of 32 MOGHE cases (56%), 126 of 379 FCDII cases (33%), 23 of 37 HMEG cases (62%), and six of 69 patients without a lesion (9%). In 146 of the 149 FCDII/HMEG cases with an identified variant (98%), a pathogenic variant in mTOR pathway genes was identified; 36 of 48 FCDI/mMCD/MOGHE cases with a variant (75%) had an *SLC35A2* variant. Six of 69 patients (9%) without a histopathological lesion also had a pathogenic variant, in either *SLC35A2* (n=5), or *DEPDC5* (n=1). The 'other' variants category contained five FCDI patients with detected *CASK*, *KRAS*, *NF1*, or *NIBPL* variants, or a 1q amplification. Two FCDII patients had an *SCN1A* variant or a 1q amplification/*PTEN* variant, and one HMEG case had a somatic uniparental disomy (UPD) of the p-arm of chromosome 16 (16p UPD). Genetic findings of individual patients with a (likely) pathogenic variant are listed in **supplementary table 1**. In 20 of 34 NL-cases (59%) and 89 of 170 patients from the pooled cohorts (52%; p-value of difference = 0.64) with a somatic pathogenic variant, a low-level variant with a variant allele frequency (VAF) < 5% was identified. Somatic variants and germline variants accounted for 29 (24%) and eight (7%) of patients in our cohort, and 162 (24%) and 33 (5%) of all patients combined, respectively. In five patients (4%) of our cohort and eight patients (2%) among all cohorts combined, a germline and (second-hit) somatic variant in the same gene was identified in the resected tissue.

Blood DNA sample analysis

In all 10 patients with a tissue VAF of ~50% whose blood DNA samples were available for analysis (eight *DEPDC5* cases, one *TSC2* case, and one *SCN1A* case), the germline variant was confirmed. In addition, in seven of 114 patients (6%) with a somatic variant in tissue and available blood DNA, mosaicism for the variant was detected in blood, with VAFs (tissue/blood %) of 24.0/2.0 % and 15.2/2.0 % in two *MTOR* cases, 4.6/2.0 %, 3.7/2.2 %, 7.0/1.0 %, and 4.4/1.0 % in four *TSC1* cases and 9/1.4 % in one *PTEN* case.

Discussion

Similar to what was previously reported (8), this study shows that a high rate of pathogenic or likely pathogenic genetic findings in HMEG (62%) as well as FCDII (33%) could be identified. Almost all variants in HMEG and FCDII involved genes comprising the mTOR signaling pathway, which is not surprising given the shared pathological features between these malformations. As reported before, genes mutated in FCDI/mMCD/MOGHE patients were related to glycosylation, the mTOR signaling pathway, or synapse or gene expression pathways, pointing toward a higher genetic heterogeneity in FCDI/mMCD/MOGHE as compared to FCDII. (13) The majority of all somatic pathogenic variants were low-level (VAF < 5%) variants. Three quarters of mMCD/FCDI/MOGHE cases with a pathogenic variant had *SLC35A2* somatic variants. In non-lesional patients the resected tissue infrequently revealed somatic, mainly *SLC35A2* variants. Blood-derived DNA analysis infrequently showed postzygotic mosaicism.

It is likely that part of the patients without an established genetic pathogenic variant are falsely negative, because a recent study reported evidence that in a substantial part of variant-negative FCDII cases, ultra-low level somatic variants in mTOR pathway genes can only be detected in pS6-enriched cells by deep WGS,[16] and because two of the pooled cohorts (USA, USA-EEBB) used

WES, which is a less sensitive approach to identify low-level somatic variants (11,13). Also, the gene panels that were used in the other cohorts (NL, Korea, France) did not include all 75 currently known MCD-related genes, and unknown genes might also play a role, although the MCD genes with the highest prevalence were analyzed.[10,12,17] Lastly, it is probable that there is an underestimation of the histopathological entity MOGHE in this study, because most of the included previously published cohorts did not yet recognize MOGHE as a distinct pathological entity during the study period. As a result, because somatic brain mosaic variants in *SCL35A2* are a major etiological factor in MOGHE, it is possible that part of the previously reported *SLC35A2* cases with a histopathological diagnosis of FCDI or normal/nonspecific histopathological findings should be classified as MOGHE under the current guidelines.[3,18]

Recent studies emphasize that genetic testing of tissue adherent to stereoelectroencephalography electrodes and cell-free DNA derived from cerebrospinal fluid collected during epilepsy surgery suggest future opportunities for detecting pathogenic variants in epilepsy surgery patients.[19,20] Further analysis of the correlation between genetic variants and surgical outcomes in cohorts such as these will improve patient counseling and may guide postoperative treatment decisions.

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Supplemental information

Supplementary table 1. List of (likely) pathogenic somatic variants identified in all 4 cohorts combined (n=166).

histopathology	center	sample	sex	gene variant	ACMG classification	somatic/ germline	brain DNA VAF (%)	blood DNA VAF (%)
HMEG	USA	dukeepi2841br	F	16pUPD	LP[a]	somatic	-	-
HMEG	KR	20	F	*MTOR* (p.Leu1460Pro)	LP/P	somatic	15.2	2.0
HMEG	KR	255	F	*MTOR* (p.Cys1483Tyr)	LP	somatic	9.8	0
HMEG	FR	HME-73	F	*MTOR* (p.Ala1459Asp)	P / LP	somatic	9.2	0
HMEG	USA	mcdbose276br	F	*MTOR* (c.G4448A; p.C1483Y)	P	somatic	11.0	-
HMEG	USA	mcdbose254br	M	*MTOR* (c.T4447C; p.C1483R)	P	somatic	24.0	2.0
HMEG	USA	dukeepi2488br	F	*MTOR* (c.T4447C; p.C1483R)	P	somatic	5.7	0
HMEG	FR	HME-74	M	*AKT3* (p.Glu17Lys b)	LP/P	somatic	12.0	0
HMEG	USA	uth0004br	M	*AKT3* (c.C863T; p.T288I)	P	somatic	44.0	0
HMEG	USA	mcdgg17015br	F	*AKT3* (c.G49A; p.E17K)	P	somatic	13.0	0
HMEG	FR	HME-75	M	*PIK3CA* (p.Glu542Lys)	LP/P	somatic	7.5	0
HMEG	FR	HME-76	F	*PIK3CA* (p.Glu542Lys)	LP/P	somatic	34.0	0
HMEG	FR	HME-77	M	*PIK3CA* (p.His1047Arg)	LP/P	somatic	21.2	0
HMEG	FR	HME-78	F	*PIK3CA* (p.His1047Arg)	LP/P	somatic	29.8	0
HMEG	KR	1407	M	*PIK3CA* (p.Glu545Lys)	P	somatic	18.6	0
HMEG	USA	COLE153BR	M	*PIK3CA* (c.A3140G; p.H1047R)	P	somatic	21.0	-
HMEG	USA	pmge174br	F	*PIK3CA* (c.G1624A; p.E542K)	P	somatic	14.0	0
HMEG	USA	mcdbose274br	F	*PIK3CA* (c.G1624A; p.E542K)	P	somatic	25.0	-
HMEG	USA	mcdbose241br	F	*PIK3CA* (c.G1633A; p.E545K)	P	somatic	28.0	-
HMEG	USA	dukeepi5208br	M	*PIK3CA* (c.G1633A; p.E545K)	P	somatic	20.0	-
HMEG	USA	mcdbose348br	M	*PIK3CA* (c.G1633A; p.E545K)	P	somatic	14.0	-
HMEG	FR	HME-79	M	*RHEB* (Tyr35Leu)	LP/P	somatic	17.6	0
HMEG	FR	FCD-36	F	*DEPDC5* (c.3021+1G>A) + LOH	LP/P	germline	65.0	48.0
FCD2	USA	pmge203br	F	1q amplification	P	somatic	-	-
FCD2	USA	pmge203br	F	*PTEN* (c.G389A; p.R130Q)	P	somatic	9.0	1.4

histopathology	center	sample	sex	gene variant	ACMG classification	somatic/ germline	brain DNA VAF (%)	blood DNA VAF (%)
FCD2b	EEBB	MCD_EEBB_85	F	*PTEN* (p.L61Nfs*2)	P	somatic	49.0	-
FCD2b	KR	112	M	*DEPDC5* (p.Phe1399fs)	LP/P	two-hit/germline	44.4	NP
FCD2b	KR	112	M	*DEPDC5* (p.Thr1508fs)	LP/P	two-hit/somatic	4.5	0
FCD2a	NL	5	M	*DEPDC5* (p.Arg286*)	LP/P	two-hit/germline	45.3	-
FCD2a	NL	5	M	*DEPDC5* (p.Glu906fs)	LP/P	two-hit/somatic	3.3	-
FCD2a	NL	30	M	*DEPDC5* (p.Asn1353fs)	LP/P	two-hit/germline	56.1	-
FCD2a	NL	30	M	*DEPDC5* (p.Arg239*)	LP/P	two-hit/somatic	3.0	-
FCD2a	FR	FCD-33	M	*DEPDC5* (p.Arg286*)	LP/P	two-hit/germline	49.0	52.0
FCD2a	FR	FCD-33	M	*DEPDC5* (p.Gln289*)	LP/P	two-hit/somatic	10.0	0
FCD2a	NL	93	M	*NPRL2* (p.Phe87Ser)	P	two-hit/germline	46.5	51.0
FCD2a	NL	93	M	*NPRL2* (p.Glu8*)	LP/P	two-hit/somatic	2.9	-
FCD2a	FR	FCD-33	M	*DEPDC5* (p.Arg286*)	LP/P	two-hit/germline	49.0	52.0
FCD2a	FR	FCD-33	M	*DEPDC5* (p.Gln289*)	LP/P	two-hit/somatic	10.0	0
FCD2a	NL	93	M	*NPRL2* (p.Phe87Ser)	P	two-hit/germline	46.5	51.0
FCD2a	NL	93	M	*NPRL2* (p.Glu8*)	LP/P	two-hit/somatic	2.9	-
FCD2b	NL	33	F	*MTOR* (p.Ser2215Tyr)	LP/P	two-hit/germline	49.3	-
FCD2b	NL	33	F	*MTOR* (p.Tyr155Cys)	LP/P	two-hit/somatic	2.2	-
FCD2b	NL	8	M	*TSC2* (p.Arg1459*)	LP/P	wo-hitgermline	48.3	0
FCD2b	NL	8	M	*TSC2* (p.Arg905Gln)	LP/P	two-hit/somatic	5.3	0
FCD2a	KR	105	M	*MTOR* (p.Arg1709His)	LP/P	somatic	1,5	0
FCD2a	KR	143	F	*MTOR* (p.Ser2215Phe)	LP/P	somatic	2.3	0
FCD2a	KR	145	F	*MTOR* (p.Thr1977Lys)	LP/P	somatic	1.5	0
FCD2a	KR	4	M	*MTOR* (p.Leu2427Pro)	P	somatic	12.6	0
FCD2a	KR	6	F	*MTOR* (p.Leu2427Pro)	P	somatic	7.3	0
FCD2a	KR	91	F	*MTOR* (p.Arg2193Cys)	LP/P	somatic	1.3	0
FCD2b	KR	107	F	*MTOR* (p.Ser2215Phe)	LP/P	somatic	2.1	0
FCD2b	KR	113	F	*MTOR* (p.Leu2427Gln)	P	somatic	2.9	0
FCD2b	KR	116	M	*MTOR* (p.Thr1977Lys)	LP/P	somatic	2.9	0
FCD2b	KR	121	M	*MTOR* (p.Tyr1450Asp)	LP/P	somatic	3.8	0
FCD2b	KR	128	F	*MTOR* (p.Cys1483Arg)	LP/P	somatic	6.6	0
FCD2a	KR	339	F	*MTOR* (p.Leu1460Pro)	LP/P	somatic	3.8	0

histopathology	center	sample	sex	gene variant	ACMG classification	somatic/ germline	brain DNA VAF (%)	blood DNA VAF (%)
FCD2a	KR	90	M	*MTOR* (p.Arg2134Trp)	LP/P	somatic	1.0	0
FCD2a	KR	93	F	*MTOR* (p.Thr1977Lys)	LP/P	somatic	1.3	0
FCD2b	KR	106	M	*MTOR* (p.Trp1456Gly)	P	somatic	1.7	0
FCD2b	KR	254	M	*MTOR* (p.Ala1459Asp)	LP/P	somatic	4.7	0
FCD2a	KR	104	M	*MTOR* (p.Arg624His)	LP/P	somatic	4.4	0
FCD2b	NL	42	M	*MTOR* (p.Ser2215Tyr)	LP/P	somatic	7.0	-
FCD2b	NL	99	M	*MTOR* (p.Cys1483Arg)	LP/P	somatic	6.0	0
FCD2a	FR	FCD-19	F	*MTOR* (p.Ala1459Asp)	LP/P	somatic	0.5	0
FCD2a	FR	FCD-20	F	*MTOR* (p.Leu1460Pro)	LP/P	somatic	18.6	0
FCD2a	FR	FCD-21	M	*MTOR* (p.Ser2215Phe)	LP/P	somatic	0.9	0
FCD2a	FR	FCD-22	M	*MTOR (*p.Ser2215Phe)	LP/P	somatic	1.2	0
FCD2a	FR	FCD-23	M	*MTOR* (p.Ser2215Phe)	LP/P	somatic	1.5-2.7	0
FCD2a	FR	FCD-24	F	*MTOR* (p.Ser2215Phe)	LP/P	somatic	2.1	0
FCD2a	FR	FCD-25	M	*MTOR (*p.Ser2215Phe)	LP/P	somatic	8.5	0
FCD2a	FR	FCD-26	F	*MTOR* (p.Ser2215Tyr)	LP/P	somatic	0.4	0
FCD2a	FR	FCD-27	M	*MTOR (*p.Ser2215Tyr)	LP/P	somatic	0.4	0
FCD2a	FR	FCD-28	M	*MTOR* (p.Ser2215Tyr)	LP/P	somatic	0.6	0
FCD2a	FR	FCD-29	M	*MTOR* (p.Ile2500Phe)	LP/P	somatic	2.3	0
FCD2b	FR	FCD-54	F	*MTOR* (p.Ala1459Asp)	LP/P	somatic	1.3-1.6	0
FCD2b	FR	FCD-55	M	*MTOR* p.Leu1460Pro)	LP/P	somatic	3.4	0
FCD2b	FR	FCD-56	F	*MTOR* (p.Thr1977Lys)	LP/P	somatic	5.5	0
FCD2b	FR	FCD-57	M	*MTOR* (p.Ser2215Phe)	LP/P	somatic	1.3	0
FCD2b	FR	FCD-58	F	*MTOR* (p.Ser2215Phe)	LP/P	somatic	1.5	0
FCD2b	FR	FCD-59	M	*MTOR* (p.Ser2215Phe)	LP/P	somatic	3.4	0
FCD2b	FR	FCD-60	F	*MTOR* (p.Ser2215Phe)	LP/P	somatic	6.5	0
FCD2b	FR	FCD-61	M	*MTOR* (p.Ser2215Tyr)	LP/P	somatic	3.7	0
FCD2b	NL	1	F	*MTOR* (p.Thr1977Lys)	LP/P	somatic	1.7	0
FCD2b	NL	28	F	*MTOR* (p.Thr1977Lys)	LP/P	somatic	4.6	-
FCD2b	NL	34	F	*MTOR* (p.Thr1977Lys)	LP/P	somatic	3.8	-
FCD2b	NL	40	M	*MTOR* (p.Ser2215Tyr)	LP/P	somatic	4.4	-

histopathology	center	sample	sex	gene variant	ACMG classification	somatic/ germline	brain DNA VAF (%)	blood DNA VAF (%)
FCD2b	NL	52	M	*MTOR* (p.Ser2215Tyr)	LP/P	somatic	4.4	-
FCD2b	NL	54	M	*MTOR* (p.Leu1460Pro)	LP/P	somatic	1.4	0
FCD2a	NL	59	F	*MTOR* (p.Thr1977Lys)	LP/P	somatic	1.4	0
FCD2a	NL	67	M	*MTOR* (p.Ile2500Phe)	LP/P	somatic	2.1	-
FCD2b	NL	69	F	*MTOR* (p.Thr1977Lys)	LP/P	somatic	1.8	-
FCD2b	NL	80	F	*MTOR* (p.Leu2427Gln)	LP/P	somatic	0.8	-
FCD2b	NL	83	F	*MTOR* (p.Leu2427Arg)	LP/P	somatic	0.7	0
FCD2	USA	MCDBOSE364BR	F	*MTOR* (c.C5930A; p.T1977K)	P	somatic	4.6	-
FCD2	USA	mcdbose252br	M	*MTOR* (c.C5930A; p.T1977K)	P	somatic	3.8	0
FCD2	USA	mcdbose244br	F	*MTOR* (c.C5930G; p.T1977R)	LP	somatic	6.1	0
FCD2	USA	mcdbose296br	F	*MTOR* (c.C6644A; p.S2215Y)	P	somatic	4.7	0
FCD2	USA	neuro1410F49br	M	*MTOR* (c.C6644A; p.S2215Y)	P	somatic	7.2	0
FCD2	USA	mcdbose316br2	M	*MTOR* (c.C6644A; p.S2215Y)	LP	somatic	1.9	0
FCD2	USA	mcdbose286br	M	*MTOR* (c.C6644T; p.S2215F)	P	somatic	5.3	-
FCD2	USA	mcdbose271br	M	*MTOR* (c.C6644T; p.S2215F)	P	somatic	4.0	0
FCD2	USA	COLE141BR	F	*MTOR* (c.C6644T; p.S2215F)	P	somatic	3.9	-
FCD2	USA	fcde183br	F	*MTOR* (c.C6644T; p.S2215F)	P	somatic	5.8	0
FCD2a	EEBB	MCD_EEBB_86	M	*MTOR* (p.C1483Y)	P	somatic	5.0	-
FCD2b	EEBB	MCD_CCF_38	M	*MTOR* (p.L1460P)	P	somatic	3.0	-
FCD2b	EEBB	MCD_CCF_53	M	*MTOR* (p.I2500N)	LP	somatic	4.0	-
FCD2b	EEBB	MCD_CCF_92	M	*MTOR* (p.I2017T)	LP	somatic	8.0	-
FCD2b	EEBB	MCD_CCF_101	F	*MTOR* (p.S2215Y)	P	somatic	3.0	-
FCD2b	EEBB	MCD_CCF_102	M	*MTOR* (p.I2500F)	LP	somatic	4.0	-
FCD2b	EEBB	MCD_EEBB_71	F	*MTOR* (p.T1977K)	P	somatic	5.0	-
FCD2b	EEBB	MCD_EEBB_80	M	*MTOR* (p.S2215F)	P	somatic	3.0	-
FCD2b	EEBB	MCD_EEBB_92	M	*MTOR* (p.P2425_L2426insP)	LP	somatic	3.0	-
FCD2a	KR	1264	M	*AKT3* (p.Glu17Lys)	P	somatic	4.4	0
FCD2a	FR	FCD-30	M	*AKT3* (p.Glu17Lys)	LP/P	somatic	1.0	-
FCD2a	FR	FCD-31	F	*AKT3* (p.Glu17Lys)	LP/P	somatic	2.0	-
FCD2a	NL	60	F	*AKT3* (p.Glu17Lys)	LP/P	somatic	12.3	-
FCD2a	NL	75	F	*AKT3* (p.Glu17Lys)	LP/P	somatic	7.0	0

histopathology	center	sample	sex	gene variant	ACMG classification	somatic/ germline	brain DNA VAF (%)	blood DNA VAF (%)
FCD2b	NL	53	M	*PTEN* (p.Pro246Leu)	LP/P	somatic	1.3	0
FCD2b	FR	FCD-62	M	*RHEB* (p.Glu40Val)	LP/P	somatic	8.9	-
FCD2a	KR	64	M	*TSC1* (p.Arg204Cys)	P	somatic	1.0	0
FCD2a	KR	81	F	*TSC1* (p.Arg22Trp)	LP/P	somatic	2.0	0
FCD2a	KR	98	M	*TSC1* (p.Arg22Trp)	LP/P	somatic	2.0	0
FCD2b	KR	123	F	*TSC1* (p.Arg22Trp)	LP/P	somatic	1.4	0
FCD2b	KR	1415	M	*TSC1* (p.Arg509*)	P	somatic	1.0	0
FCD2b	KR	164	M	*TSC1* (p.Arg692*)	P	somatic	3.9	0
FCD2a	KR	94	F	*TSC2* (p.Val1547Ile)	LP/P	somatic	1.6	0
FCD2b	FR	FCD-63	F	*TSC1* (p.Arg509*)	LP/P	somatic	4.6	2.0
FCD2b	FR	FCD-64	M	*TSC1* (p.Glu636fs*51)	LP/P	somatic	3.7	2.2
FCD2b	NL	19	M	*TSC1* (p.Asn891fs)	LP/P	somatic	4.1	0
FCD2b	NL	58	F	*TSC1* (p.Q842*)	LP/P	somatic	7.0	-
FCD2	USA	fcde191br	F	*TSC1* (c.C298T; p.Q100X)	P	somatic	4.4	1.0
FCD2b	FR	FCD-65	M	*TSC2* (p.Arg1743Gln)	LP/P	somatic	1.0	0
FCD2b	FR	FCD-66	M	*TSC2* (p.Gln794*)	LP/P	somatic	1.5	0
FCD2b	NL	4	M	*TSC2* (p.Gln1178*)	LP/P	somatic	4.3	-
FCD2	USA	uth0016br	F	*TSC2* (c.1934_1935insCTGCGAC: p.Y648Lfs*11)	P	somatic	1.7	0
FCD2b	EEBB	MCD_CCF_49	F	*MTOR* (p.W1916C)	LP	germline	46.0	-
FCD2	USA	mcdbose247br	M	*SCN1A* (c.T662C; p.L221P)	P	germline	46.0	42.0
FCD2b	KR	101	M	*DEPDC5* (p.Arg1268*)	P	germline	48.9	NP
FCD2b	KR	250	F	*DEPDC5* (p.Arg1136*)	LP/P	germline	50.0	NP
FCD2b	KR	70	F	*DEPDC5* (p.Gln372*)	P	germline	50.6	NP
FCD2a	NL	97	M	*DEPDC5* (c.871+1G>T)	P	germline	57.0	49.0
FCD2a	FR	FCD-34	M	*DEPDC5* (c.279+1G>A	LP/P	germline	49.0	47.0
FCD2a	FR	FCD-35	M	*DEPDC5* (p.Arg239*)	LP/P	germline	51.0	49.0
FCD2a	FR	FCD-37	F	*DEPDC5* (p.Glu1385fs)	LP/P	germline	53.0.	49.0
FCD2a	NL	15	M	*DEPDC5* (p.Tyr1537fs)	LP/P	germline	52.4	-
FCD2a	NL	76	F	*DEPDC5* (p.Arg874*)	P	germline	48.9	51.0
FCD2a	NL	88	M	*DEPDC5* (p.Arg555*)	LP/P	germline	46.8	-

histopathology	center	sample	sex	gene variant	ACMG classification	somatic/ germline	brain DNA VAF (%)	blood DNA VAF (%)
FCD2a	KR	79	M	*DEPDC5* (p.Trp1213*)	P	germline	49.0	NP
FCD2	USA	mcdbose298br	F	*DEPDC5* (p.Arg286*)	P	germline	50.0	49.0
FCD2a	EEBB	MCD_EEBB_75	M	*DEPDC5* (p.R874X)	P	germline	43.0	-
FCD2a	EBBB	MCD_EEBB_91	M	*DEPDC5* (p.R239X)	P	germline	42.0	-
FCD2a	EEBB	MCD_CCF_13	F	*NPRL2* (p.Q188X)	P	germline	53.0	-
FCD2a	EBBB	MCD_CCF_60	F	*NPRL2* (p.Y25Lfs*5)	P	germline	57.0	-
FCD2a	NL	43	F	*NPRL3* (p.Leu142fs)	P	germline	50.0	51.0
FCD2a	EEBB	MCD_CCF_46	F	*NPRL3* (p.A384Rfs*71)	P	germline	50.0	-
FCD2a	EEBB	MCD_CCF_58	F	*NPRL3* (p.W319X)	P	germline	56.0	-
FCD2a	EEBB	MCD_CCF_83	F	*NPRL3* (p.M529Cfs*23)	P	germline	48.0	-
FCD2a	FR	FCD-32	F	*TSC2* (p.Glu1243fs)	LP/P	germline	51.0	49.0
FCD2b	NL	3	F	*TSC2* (p.Pro1781fs)	LP/P	germline	25.8	-
FCD2b	EEBB	MCD_CCF_28	F	*TSC2* (p.W82R)	LP	germline	48.0	-
FCD2b	EEBB	MCD_CCF_48	F	*TSC2* (p.M740I)	LP	germline	48.0	-
FCD1	USA	uth0008br	M	1q amplification	P	somatic	-	-
FCD1	USA	mcdbose208br	F	*CASK* (c.2302 + 1G > A)	P	somatic	6.5	0
FCD1	USA	mcdbose325br	M	*KRAS* (c.G35T; p.G12V)	P	somatic	20	-
FCD1	USA	COLE160BR	M	*NF1* (c.1017_1018del;p.S340Cfs*12)	P	somatic	3.0	0
FCD1	USA	uth0005br	F	*NIPBL* (c.7685 + 1G >A)	LP	somatic	6.7	0
FCD1	USA	mcdbose240br	M	*SLC35A2* (c.757_758insGCTCTGGTGGG: p.A253Gfs*100)	P	somatic	25	0
FCD1	USA	mcdbose269br	M	*SLC35A2 (*c.C547T; p.Q183X)	P	somatic	1.9	0
FCD1	USA	MCD19035BR	M	*SLC35A2 (*c.C435A: p.Y145X)	P	somatic	27	-
FCD1	USA	mcdbose233br	M	*SLC35A2* (c.G164T: p.R55L)	LP	somatic	54	0
mMCD	KR	13	F	*SLC35A2* (p.Asn235Gln)	LP/P	somatic	10	0
mMCD	KR	150	M	*SLC35A2* (p.Glu254*)	LP/P	somatic	16	0
mMCD	KR	219	M	*SLC35A2* (p.Gln197*)	LP/P	somatic	23	0
mMCD	KR	1428	M	*SLC35A2* (p.Gly309Asp)	LP/P	somatic	3.7	0
mMCD	NL	95	F	*SLC35A2*	LP/P	somatic	NP	-
mMCD2	FR	FCD-1	M	*SLC35A2* (p.Tyr267*)	LP/P	somatic	12.1	0

histopathology	center	sample	sex	gene variant	ACMG classification	somatic/ germline	brain DNA VAF (%)	blood DNA VAF (%)
mMCD	KR	340	M	*SLC35A2* (p.Gln168*)	LP/P	somatic	18.0	0
mMCD2	FR	FCD-3	M	*SLC35A2* (p.Leu296del)	LP/P	somatic	22.4	0
mMCD2	FR	FCD-2	M	*SLC35A2* (p.Ser212Leufs*9)	LP/P	somatic	22.6	0
mMCD	NL	22	F	*mTOR* (p.Cys1483Tyr)	LP/P	somatic	4.0	-
mMCD	NL	21	M	*SLC35A2* (p.Gly282Arg)	LP/P	somatic	3.5	0
mMCD	NL	77	F	*TSC1* (p.Thr1159Pro)	LP/P	somatic	4.5	-
mMCD2	FR	FCD-4	M	*SLC35A2* (p.Pro269Thrfs*24)	LP/P	somatic	43.0	0
mMCD	NL	65	F	*SLC35A2* (p.Gln185*)	LP/P	somatic	6.0	0
mMCD	NL	12	F	*SLC35A2* (p.Gly190fs)	LP/P	somatic	7.7	0
mMCD	NL	66	F	*SLC35A2* (p.Ser212fs)	LP/P	somatic	8.6	0
mMCD	NL	49	F	*MTOR* (p.Asp1458dup)	LP/P	somatic	9.1	-
MOGHE	NL	39	M	*SLC35A2* (p.Leu86fs)	LP/P	somatic	17.8	0
MOGHE	EEBB	MCD_CCF_19	M	*SLC35A2* (p.L211Pfs*137)	P	somatic	5.0	-
MOGHE	EEBB	MCD_CCF_43	M	*SLC35A2* (p.C210Y)	LP	somatic	5.0	-
MOGHE	EEBB	MCD_CCF_79	F	*SLC35A2* (p.A116E)	LP	somatic	14.0	-
MOGHE	EEBB	MCD_CCF_108	M	*SLC35A2* (p.F65Pfs*30)	P	somatic	24.0	-
MOGHE	EEBB	MCD_EEBB_2	F	*SLC35A2* (p.S312F)	LP	somatic	8.0	-
MOGHE	EEBB	MCD_EEBB_17	F	*SLC35A2* (p.L172P)	LP	somatic	3.0	-
MOGHE	EEBB	MCD_EEBB_34	F	*SLC35A2* (p.Y122_T123insL)	LP	somatic	9.0	-
MOGHE	EEBB	MCD_EEBB_34	F	*SLC35A2* (p.Y122Sfs*6)	P	somatic	15.0	-
MOGHE	EEBB	MCD_EEBB_37	M	*SLC35A2* (p.K222del)	LP	somatic	29.0	-
MOGHE	EEBB	MCD_EEBB_58	M	*SLC35A2* (p.S308Wfs*106)	P	somatic	11.0	-
MOGHE	EEBB	MCD_EEBB_63	M	*SLC35A2* (p.G226Rfs*29)	P	somatic	4.0	-
MOGHE	EEBB	MCD_EEBB_69	F	*SLC35A2* (p.G214R)	LP	somatic	8.0	-
MOGHE	EEBB	MCD_EEBB_87	F	*SLC35A2* (p.Q108X)	P	somatic	9.0	-
MOGHE	EEBB	MCD_EEBB_97	F	*SLC35A2* (p.S302F)	LP	somatic	8.0	-
MOGHE	EEBB	MCD_EEBB_100	M	*SLC35A2* (p.Q108X)	P	somatic	25.0	-
MOGHE	EEBB	MCD_EEBB_102	F	*SLC35A2* (p.S312F)	LP	somatic	12.0	-
MOGHE	EEBB	MCD_EEBB_106	M	*SLC35A2* (p.Q278X)	P	somatic	48.0	-
FCD1	USA	mcdbose362br	M	*DEPDC5* (c.C1759T:p.R587X)	P	germline	50	-
mMCD	KR	14	M	*DEPDC5* (c.3021+1G>A)	LP/P	germline	52.1	NP
mMCD	NL	50	F	*TSC1* (p.Gln55*)	LP/P	germline	46.0	-

histopathology	center	sample	sex	gene variant	ACMG classification	somatic/ germline	brain DNA VAF (%)	blood DNA VAF (%)
mMCD	NL	37	M	*DEPDC5* (p.Arg1323*)	P	germline	47.4	49.0
No lesion	KR	44	M	*SLC35A2* (X:43763821 C>A)	LP/P	somatic	5.0	0
No lesion	KR	147	F	*SLC35A2* (p.Gln185*)	LP/P	somatic	6.0	0
No lesion	KR	395	M	*SLC35A2* (p.Leu148Pro)	LP/P	somatic	1.4	0
No lesion	KR	1429	F	*SLC35A2* (p.Leu252Pro)	LP/P	somatic	3.7	0
No lesion	NL	511	F	*SLC35A2* (p.W252*)	LP/P	somatic	15.0	-
No lesion	KR	1422	F	*DEPDC5* (c.1324+1G>A)	LP/P	germline	51.2	NP

LP = likely pathogenic; P = pathogenic; NP = not provided

Supplementary table 2. List of genes included in the sequencing panels used for genetic testing per center.

center	panel	gene number	genes types
NL	1	18	*DEPDC5, NPRL2, NPRL3, AKT1, AKT3, MTOR, PIK3CA, PIK3R1, PIK3R2, PTEN, RHEB, BRAF, TSC1, TSC2, TBC1D, RPS6, SLC35A2, FGFR1*
	2[a]	16	*AKT1, AKT3, BRAF, DEPDC5, FGFR1, MTOR, NPRL2, NPRL3, PIK3CA, PIK3R2, PTEN, RYR3, TBC1D7, TSC1, TSC2, SLC35A2*
KR	1	28	*MTOR, AKT3, PIK3CA, PIK3R2, PTEN, STRADA, TSC1, TSC2, DEPDC5, SCN1A, CACNA1A, GRIN1, GABRA1, GABRB3, GABBR2, DCX, CHD2, LIS1, TUBB2B, FASN, FLNA, BRAF, EZH2, HNRNPU, IQSEC2, NEDD4L, ALG13, DNM1*
	2	13	*MTOR, AKT3, AKT1, PIK3CA, PIK3R2, DEPDC5, TSC1, TSC2, NPRL2, NPRL3, PTEN, BRAF, SLC35A2*
FR	1	25	*AKT1*, *AKT2*, *AKT3, CNTNAP2*), *DEPDC5*, *MIOS*, *MTOR*, *NPRL2*, *NPRL3*, *PIK3CA*, *PIK3CB*, *PIK3CD*, *PIK3R1*, *PIK3R2*, *PIK3R3*, *PTEN*, *RAB3GAP1*, *SEC13*, *SEH1L*, *STRADA*, *TBC1D7*, *TSC1*, *TSC2*, *WDR24*, *WDR59*
	2	30	*AKT3*, *BRAF*, *DEPDC5*, *DEPTOR*, *FLCN*, *LAMTOR4*, *LARS*, *MIOS*, *MLST8*, *MTOR*, *NPRL2*, *NPRL3*, *PIK3CA*, *PIK3R2*, *PTEN*, *RABGAP1L*, *RHEB*, *RPS6*, *RPS6KB*, *RPTOR*, *RRAGA*, *RRAGAB*, *SEC13*, *SEH1L*, *SESN3*, *SLC13A5*, *TSC1*, *TSC2*, *WDR24*, *WDR59 AKT3*, *BRAF*, *DEPDC5*, *DEPTOR*, *LAMTOR4*, *MIOS*, *MLST8*, *MTOR*, *NPRL2*, *NPRL3*, *PIK3CA*, *PTEN*, *RABGAP1L*, *RHEB*, *RICTOR*, *RPS6*, *RPTOR*, *SEC13*, *SEH1L*, *SLC35A2*, *SZT2*, *TBC1D7*, *TSC1*, *TSC2*, *WDR24*, *WDR59*
	3	26	*AKT3, BRAF, DEPDC5, DEPTOR, LAMTOR4, MIOS, MLST8, MTOR, NPRL2, NPRL3, PIK3CA, PTEN, RABGAP1L, RHEB, RICTOR (NM_001285439.1), RPS6, RPTOR, SEC13, SEH1L, SLC35A2 (NM_005660.2), SZT2 (NM_015284.3), TBC1D7, TSC1, TSC2, WDR24, WDR59*

center	panel	gene number	genes types
USA[b]	N/A	N/A	N/A
USA-EEBB[b]	N/A	N/A	N/A

[a] panel 2 was performed in all NL patients in whom the results of panel 1 were negative
[b] all individuals from the USA cohort underwent exome sequencing

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Part III

7

Outcome of epilepsy surgery in MRI-negative patients without histopathologic abnormalities in the resected tissue

Maurits W.C.B. Sanders, Iskander Van der Wolf, Floor E. Jansen, Eleonora Aronica, Christoph Helmstaedter, Attila Racz, Rainer Surges, Alexander Grote, Albert J. Becker, Sylvain Rheims, Hélène Catenoix, John S. Duncan, Jane De Tisi, Thomas S. Jacques, J. Helen Cross, Reetta Kalviainen, Tuomas Rauramaa, Francine Chassoux, Bertrand C. Devaux, Giancarlo Di Gennaro, Vincenzo Esposito, Istvan Bodi, Mrinalini Honavar, Christian G. Bien, Thomas Cloppenborg, Roland Coras, Hajo M. Hamer, Petr Marusic, Adam Kalina, Tom Pieper, Manfred Kudernatsch, Till S. Hartlieb, Tim J. von Oertzen, Martin Aichholzer, Georg Dorfmüller, Mathilde Chipaux, Soheyl Noachtar, Elisabeth Kaufmann, Andreas Schulze-Bonhage, Christian F. Scheiwe, Cigdem Özkara, Thomas Grunwald, Kristina Koenig, Renzo Guerrini, Carmen Barba, Anna Maria Buccoliero, Flavio Giordano, Felix Rosenow, Katja Menzler, Rita Garbelli, Francesco Deleo, Pavel Krsek, Barbora Straka, Alexis A. Arzimanoglou, Joseph Toulouse, Wim Van Paesschen, Tom Theys, José Pimentel, Isabel M. Loução De Amorim, Nicola Specchio, Luca De Palma, Martha Feucht, Theresa Scholl, Karl Roessler, Rafael Toledano Delgado, Antonio Gil-Nagel, Savo Raicevic, Aleksandar J. Ristic, Olaf Schijns, Jan Beckervordersandforth, Victoria San Antonio-Arce, Jordi Rumia, Ingmar Blümcke*, Kees P.J. Braun*

*These authors contributed equally

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ABSTRACT

Objective

Patients with presumed non-lesional focal epilepsy (based on either MRI or histopathological findings) have a lower success rate of epilepsy surgery compared to lesional patients. Here, we aimed to characterize a large group of focal epilepsy patients who underwent epilepsy surgery despite a normal MRI and had no lesion on histopathology. Determinants of their postoperative seizure outcomes were further studied.

Methods

We designed an observational multicenter cohort study of MRI- and histopathology-negative patients who were derived from the European Epilepsy Brain Bank and underwent epilepsy surgery between 2000 and 2012 in 34 epilepsy surgery centers within Europe. We collected data on clinical characteristics, presurgical assessment (including genetic testing), surgery characteristics, postoperative outcome and treatment regimen.

Results

Of the 217 included patients, 40% were seizure-free (Engel I) 2 years after surgery and one-third of patients remained seizure-free after 5 years. Temporal lobe surgery (adjusted odds ratio (AOR): 2.62; 95%-CI, 1.19–5.76), shorter epilepsy duration (AOR for duration: 0.94; 95%-CI, 0.89–0.99), and completely normal histopathological findings (versus non-specific reactive gliosis) (AOR: 4.69; 95%-CI, 1.79–11.27) were significantly associated with favorable seizure outcome at 2 years after surgery. Of patients who underwent invasive monitoring, only 35% reached seizure-freedom at 2 years. Patients with parietal lobe resections had lowest seizure-freedom rates (12.5%). Amongst temporal lobe surgery patients, there was a trend towards favorable outcome if hippocampectomy was part of the resection strategy (OR: 2.94; 95%-CI, 0.98–8.80). Genetic testing was only sporadically performed.

Discussion

This study shows that seizure-freedom can be reached in 40% of non-lesional patients with both normal MRI and normal histopathology findings. In particular non-lesional temporal lobe epilepsy (TLE) should be regarded as a relatively favorable group, with almost half of patients achieving seizure-freedom at 2 years after surgery (even more if the hippocampus is resected) compared to only 1 in 5 non-lesional patients who underwent extratemporal surgery. Patients with an electroclinically identified focus, who are non-lesional, will be a promising group for advanced molecular-genetic analysis of brain tissue specimens to identify new brain somatic epilepsy genes or epilepsy-associated molecular pathways.

Introduction

Epilepsy surgery is a successful treatment option for patients with medically refractory focal epilepsy with an average 2-year postoperative seizure-freedom rate of 67.5%.[1,2] Patients are preferably considered for surgery when the epileptogenic zone is focal, of presumed structural origin, well-delineated, and outside eloquent areas. Excluding palliative procedures, epilepsy surgery aims to completely remove or disconnect the epileptogenic zone.[3] Although the absence of a MRI-detected lesion is associated with a less favorable surgical outcome,[1,4] presurgical evaluation in MRI-negative patients with focal epilepsy is justified, acknowledging that a considerable proportion of those patients have a structural etiology, particularly a subtype of focal cortical dysplasia (FCD) or mild malformation of cortical development (mMCD). A prerequisite for surgical candidacy is a consistent hypothesis about the seizure onset zone, based on multimodal diagnostic noninvasive functional imaging and source localization techniques, often followed by invasive monitoring (subdural grid implantation or stereo-EEG).[5] In addition, genetic testing could improve the selection of eligible surgery candidates,[6] as it is increasingly acknowledged that many patients with focal epilepsy have an underlying genetic etiology that may render them less or more suitable surgical candidates, depending on the gene involved.[6,7]

Outcomes of epilepsy across all histopathological categories in a large cohort of patients in Europe (the European Epilepsy Brain Bank) were recently reported.[1,8] In almost 8% of the reviewed brain tissues, no specific pathological diagnosis could be identified. The absence of a specific histopathological diagnosis can theoretically be the consequence of a surgical strategy in which the lesion itself is not or insufficiently sampled for pathological evaluation (i.e., a sample error), which would include the consequences of certain surgical (e.g., aspiration) techniques that do not allow tissue specimens to be taken, or the lesion is disconnected rather than resected. In these scenarios, the epilepsy can still be lesional, as evidenced by an abnormal MRI. Alternatively, especially in the absence of an MRI-visible lesion, histopathology may be normal because the epilepsy is truly non-lesional, and caused by mechanisms that cannot be detected with microscopic testing, such as genetic etiologies causing focal epilepsy.[9,10]

Similar to the relatively poor outcome of surgery in patients with normal MRI-findings, normal or non-specific histopathological evaluation was also related to less favorable surgical outcomes; the proportion of seizure-free patients in the subgroup of patients in whom histopathological testing of resected brain tissue revealed no, or no specific lesion, was 60%, 54% and 51% after 1, 2 and 5 years respectively, versus 73%, 69%, and 68% in the lesional group.[1]

Although some studies pointed out the potential benefits of surgery even in non-lesional forms of focal epilepsy,[9] a substantial part of these patients is unnecessarily exposed to the risks of invasive presurgical assessment and surgery, as they experience recurrent seizures after surgery. Whereas several studies have reported and reviewed the outcomes of MRI-negative epilepsy patients[10-12], little is known about the presurgical assessment strategies and postsurgical outcomes of epilepsy patients who are considered to be 'truly' non-lesional, based on both imaging and histopathological findings.

We conducted this observational cohort study to evaluate the presurgical trajectory, surgery characteristics and postoperative outcome in MRI-negative focal epilepsy patients in whom also histopathological examination of resected brain tissue showed no specific abnormalities. We hypothesized that outcomes of these patients are worse compared to those with normal pathology but a likely epileptogenic MRI lesion. Lastly, we assessed determinants of postoperative outcome in this challenging group of non-lesional patients.

Methods

Patient selection

We retrospectively evaluated data from the pre- and postsurgical trajectories in a cohort of 836 pathology-negative patients derived from the European Epilepsy Brain Bank (EEBB) cohort, that included a total of 9147 operated patients from 34 epilepsy surgery centers across 14 countries.[1] All patients underwent epilepsy surgery between 2000 and 2012. We included patients of any age, in whom histopathological examination of the resected brain tissue revealed no abnormalities, or only mild non-specific reactive gliosis, therefore not demonstrating a specific structural etiology (thus not in the context of e.g. a vascular lesion).

First, we contacted collaborators from the EEBB consortium to retrospectively collect additional data from the patients who were categorized in this pathology-negative cohort.[1] Second, from the patients whose complete datasets were acquired, we identified a subgroup of patients in whom MR-imaging strongly suggested a structural epileptogenic lesion; notwithstanding the absence of histopathological abnormalities. In these individuals, histopathological results were considered to be non-representative and falsely negative. In the remaining group, both MRI and histopathology revealed no epileptogenic lesion, and the epilepsy was considered to be non-lesional.

Standard protocol approvals, registrations, and patient consents

The study was approved by the University of Erlangen ethical review board (193_18b for EEBB of the European Reference Network 'EpiCare'). De-identified data sets were submitted from local epilepsy centers to the EEBB database in Erlangen, Germany, using electronic data sheets (Excel). Informed consent was obtained from all patients at the submitting center and all procedures were performed in accordance with the ethics requirements of the contributing center.

Data collection

Our collaborators retrieved information on the clinical course of the patients' epilepsies, presurgical diagnostic trajectories, surgeries, and their postsurgical outcomes retrospectively from the patients' health records. Clinical variables included age of epilepsy onset, disease duration until epilepsy surgery, age at surgery, family history of epilepsy, comorbidities and the presurgical presumed etiological diagnosis. Information on the presurgical diagnostic evaluation included: duration of the presurgical trajectory as defined by the duration from start of the evaluation program until surgery, results of MRI as reported by a dedicated neuroradiologist in the participating center, highest magnetic field strength used, use of video-electroencephalography monitoring (V-EEG), invasive intracranial electroencephalography (iEEG), positron emission tomography (PET), single photon emission computed tomography (SPECT), and magnetoencephalography (MEG). Furthermore, types and results of genetic testing, if performed, either before or after surgery, were collected.

Surgical procedures were categorized as 1) hemispherotomy, 2) (multi-)lobar resection, 3) focal resection, and 4) disconnection (other than hemispherotomy). Moreover, we collected side and location of surgery, as well as (expected) completeness of the resection, as documented with perioperative electrocorticography or based on preresection iEEG findings. Outcome reports of histopathological examination were reviewed by a dedicated pathologist and results were characterized as 1) completely normal, or 2) only non-specific reactive changes, such as mild gliosis, not indicative of a structural epilepsy etiology. In our previous study, detailed information on seizure-freedom (defined as Engel I as well as postoperative pharmacological treatment regimen

(freedom of antiseizure medication) at three different time points (1, 2 and 5 years after surgery) had already been collected.[13]

Analysis

Differences in baseline characteristics between the likely non-lesional versus the MRI-lesional group were examined by use of χ^2 tests for categorical variables and Mann-Whitney or independent-sample t-tests for continuous variables. Categorical data are presented as percentages, and continuous variables as median and mean values. As a primary analysis, we performed univariable and multivariable regression analysis in the non-lesional cohort to calculate (adjusted) odds ratios in patients who were rendered seizure-free compared to those with recurrent seizures after surgery, associated with the following available and possibly relevant determinants of seizure outcome: age of surgery and duration of epilepsy, surgery location (temporal versus extratemporal), use of invasive diagnostics, and histopathological diagnosis (normal findings versus non-specific reactive gliosis). Due to high collinearities, completeness of resection was not included in the multivariable model. Data analysis was performed with IBM SPSS 23.

Results

After contacting the treating physicians from the 34 epilepsy surgery centers of the original EEBB cohort,[1] we were able to retrieve additional data of 706 of 836 (84%) eligible pathology-negative patients from 31 centers (**supplementary table 1**). In 571 of 706 patients (83%), the data were sufficient to draw conclusions on the presence or absence of a structural cause. Disease characteristics – other than age at epilepsy onset and surgery– and surgical outcomes did not differ between the 571 included patients and the remaining 265 of whom data was incomplete or could not be retrieved (**supplementary table 2)**.

Of these 571 patients, 354 (62%) had evidence of a causative MRI-lesion. Their MRI-findings, and thus imaging-based presumed structural etiologies, are summarized in **supplementary table 3** The remaining 217 MRI-negative patients (38%) with normal histopathological examination were considered to be non-lesional.

Table 1. Baseline characteristics of patients with complete data (N=571).

CHARACTERISTICS	COHORT	NON-LESIONAL (n=217)	MRI-LESIONAL (n=354)	P-VALUE OF DIFFERENCE
epilepsy trajectory	age onset – yr, mean (SD)	15.0 (± 10)	12.7 (± 11)	0.02
	male sex – no. (%)	105 (48)	213 (60)	0.02
	age surgery – yr, mean (SD)	32.8 (± 12)	28.2 (±15)	0.00
	MRI field strength ³ 1.5T – no. (%)	175 (81)	297 (84)	0.62
presurgical trajectory	duration presurgical trajectory – mo. median (IQR)	18 (9-36)	14 (6-40)	0.04
	PET – no. (%)	157 (71)	109 (31)	0.00
	SPECT – no. (%)	50 (23)	50 (14)	0.01
	MEG – no. (%)	25 (12)	22 (6)	0.02
	HD-EEG – no. (%)	14 (6)	45 (13)	0.02
	iEEG – no. (%)	158 (73)	57 (16)	0.00
	genetic testing – no. (%)	15 (7)	16 (5)	0.20
surgery	surgery location			
	temporal – no. (%)	150 (69)	199 (56)	0.01
	frontal – no. (%)	40 (18)	59 (17)	0.59
	parietal – no. (%)	11 (5)	10 (3)	0.31
	occipital – no. (%)	3 (2)	8 (2)	0.26
	multilobar – no. (%)	13 (6)	78 (22)	0.00
	surgery type			
	resective – no. (%)	213 (98)	293 (83)	0.00
	disconnective/hemispherotomy – no. (%)	4 (2)	61 (13)	0.00

Patient characteristics and presurgical trajectory

Clinical characteristics of the 571 histopathology-negative patients with complete data are summarized in **table 1**. The majority of variables significantly differed between the 217 non-lesional and 354 lesional patients, except for highest MRI field strength used, frequency of genetic testing, and location of surgery. The mean age at epilepsy onset was significantly higher in the non-lesional group (14.98 yr.) when compared to the MRI-lesional cases (12.73 yr.), as was age at surgery (32.8 yr. versus 28.2 yr.) and mean duration of the presurgical trajectory (18 versus 15 months). At the time of surgery, 24 (11%) of non-lesional patients were children (<18 yr.), compared to 104 (29%) of the lesional cases. One hundred sixty-four non-lesional patients (76%) underwent additional imaging modalities (PET, SPECT) or source localization techniques (MEG, HD-EEG; high-density electroencephalography). Non-lesional patients significantly more often underwent invasive monitoring (73% versus 16%).

Any form of genetic testing –on blood or tissue, and either before or after surgery– at the time of data collection was performed in only 15 (7%) of non-lesional patients and 16 (5%) of patients with a structural cause of epilepsy. None of the 16 genetic tests performed in the 15 non-lesional patients yielded a causative variant. In the 26 tests done in the 16 lesional patients, one likely pathogenic germline variant (*CCM2)* and 3 germline variants of unknown significance were identified (**supplementary table 4)**.

Table 2. Surgery outcome in non-lesional temporal *versus* extratemporal cases: non-invasive *versus* invasive monitoring; normal histopathological findings *versus* non-specific reactive gliosis.

SEIZURE OUTCOME / NON-LESIONAL COHORT	N	ENGEL I 1YR (%)	ENGEL I 2YR (%)	ENGEL I 5 YR (%)
all	**217**	**47.4**	**40.0**	**36.3**
temporal	**150**	**58.1**	**47.3**	**41.0**
no IEEG	50	71.4	60.9	50.0
IEEG	100	51.5	41.1	36.4
normal histopathological findings	38	57.9	55.6	44.1
non-specific reactive gliosis	112	58.1	44.2	38.8
hippocampectomy	60[a]	63.3	54.0	52.0
no hippocampectomy	23[a]	39.1	28.5	25.0
extratemporal	**67**	**22.4**	**20.9**	**16.4**
no IEEG	10	30.0	20.0	20.0
IEEG	57	21.1	21.1	15.7
normal histopathological findings	28	35.7	32.0	25.0
non-specific reactive gliosis	39	12.8	12.8	12.8

[a] no definitive data on type of surgery (hippocampectomy yes or no) could be retrieved in 67 patients

Surgery characteristics and postoperative seizure outcomes

Fifty-four of the 217 non-lesional patients (25%) underwent surgery in either the frontal, parietal or occipital lobe. The majority of patients underwent temporal lobe surgery, comprising 150 (69%) of non-lesional epilepsy patients, and in 13 patients (6%) the location was considered multilobar. Two percent of non-lesional surgical cases underwent (hemispheric) disconnective surgery.

Postoperative seizure outcome data were not available in 1% of non-lesional cases at 1 year, 12 % of cases at 2 years, and 43% of patients at 5 years. Engel I seizure-freedom rates at 1, 2 and 5 years after surgery in patients with non-lesional epilepsy are listed in **table 2.** All other seizure outcome rates (Engel I to IV) are listed in **supplementary table 5**. On average, 40% was seizure-free after 2 years, ranging from 20.9% in extratemporal to 47.3% in temporal lobe surgery. Seizure-freedom in combination with complete tapering of anti-seizure medication was achieved in 17.2% of patients at 2 years of follow-up. Univariably, longer duration of epilepsy, extratemporal surgery, long-term invasive monitoring, incomplete resection, and non-specific reactive gliosis (versus completely normal histopathological findings) were significantly associated with a lower chance of reaching seizure-freedom at 2 years (**table 3**). Amongst patients who underwent temporal lobe surgery,

there was a trend towards favorable outcome in patients of whom resection included hippocampectomy (OR: 2.94; 95%-CI, 0.98–8.80). Multivariable analysis showed a significant association between seizure-freedom at 2 years and temporal surgery (adjusted odds-ratio (AOR): 2.62; 95%-CI 1.19–5.76), shorter epilepsy duration (AOR for duration: 0.94; 95%-CI, 0.89–0.99), and completely normal histopathological findings (AOR: 4.49; 95%-CI, 1.79–11.27). Of the non-lesional patients who were seizure-free at 1 year after surgery, 23.4% lost seizure-freedom at later follow-up.
In patients with MRI-lesional epilepsy, seizure outcomes were better than in non-lesional cases, with an inverse odds ratio of 0.39 of being seizure-free 1 year following surgery in non-lesional epilepsy patients compared to MRI-lesional cases, who had seizure-freedom rates of 70%, 62%, and 60% at 1, 2 and 5 years following surgery (**supplementary table 6)**.

Table 3. Multivariate regression analysis in non-lesional patients with favorable *versus* unfavorable outcome.

SEIZURE OUTCOME / CHARACTERISTICS	ENGEL I[a] (n=76)	ENGEL II-IV[a] (n= 114)	P-VALUE OF DIFFERENCE	UNIVARIABLE ODDS RATI (95% CI)	MULTIVARIABLE ODDS RATIO (95% CI)[b]
age surgery – yr, mean (SD)	32.1(± 12.0)	32.1 (± 12.5)	0.98	1.00 (0.98–1.02)[c]	1.04 (0.99–1.09)
duration epilepsy – yr, median (IQR)	14.5 (8.1-21.5)	19 (9.0-26.8)	0.05	0.97 (0.94–0.99)[c]	0.94 (0.89–0.99)
temporal surgery location – no. (%)	62 (82)	69 (60)	0.00	2.89 (1.45–5.76)	2.62 (1.19–5.76)
iEEG – no. (%)	49 (64)	93 (82)	0.01	0.41 (0.21–0.80)	0.45 (0.17–1.22)
complete resection – no. (%)	39/46 (85)[d]	47/81 (58)[e]	0.00	4.03 (1.61–10.09)	- [f]
normal histopathological findings – no. (%, *versus* non-specific gliosis)	28 (37)	33 (29)	0.25	1.43 (0.77–2.65)	4.49 (1.79–11.27)

[a] Engel classification at 2 years follow-up, 27 missing cases (12%)
[b] adjusted for all other covariates
[c] odds ratio per year
[d] no exact information on completeness of resection (n=28), no resective surgery (n=2)
[e] no exact information on completeness of resection (n=32), no resective surgery (n=1)
[f] not included due to high collinearities

Discussion

This study presents a cohort of 217 European epilepsy patients who were considered truly non-lesional, having neither imaging nor histopathological abnormalities pointing towards a structural epilepsy cause. Their seizure-outcome was remarkably poor, with little more than one-third of non-lesional patients being seizure-free 5 years following surgery and one fifth of patients achieving seizure-freedom when considering extratemporal surgery only. However, non-lesional patients who underwent temporal lobe surgery had a (statistically significant) more favorable outcome, with almost half of patients achieving seizure-freedom 2 years postoperatively. Patients in whom surgery also involved resection of the hippocampus experienced even better postoperative results: more than half of patients in this subcategory were rendered seizure-free at 2 as well as 5 years following surgery. Longer duration of epilepsy and non-specific histopathological abnormalities each were independently related to poorer outcome.

Most previous studies on surgical outcomes in so-called 'non-lesional' epilepsy referred to the absence of either an MRI-visible epileptogenic lesion, or abnormal histopathological findings not combined with imaging findings. A systematic review on surgical outcomes in such MRI-non-lesional epilepsy reported seizure-freedom in 46% of 398 patients, without specifying duration of seizure-freedom, and in 39% of 302 non-lesional patients when considering only histopathology.[14] In a substantial part of the MRI-negative surgical patients, however, histopathological examination reveals a structural cause of epilepsy, particularly subtypes of FCD.[15,16] One retrospective review addressed outcomes of surgical patients who were non-lesional from a histopathological perspective, and showed a seizure-freedom rate of 42.9% of 21 patients, after a mean follow-up time of 6.5 years.[9] We are aware of only one other relatively small study that evaluated seizure outcome specifically in patients who underwent temporal lobe epilepsy (TLE) surgery and had both normal MRI and microscopic examination, that reported seizure-freedom in 61.5% of 26 patients 2 years following surgery.[17]

Although for the entire group of MRI- and pathology-negative patients, long-term seizure-freedom rates were disappointing, this study underlines that in a specific group of patients without a structural cause of epilepsy, comprising of candidates for temporal lobe surgery and mesiotemporal surgery in particular, resective surgery is a viable treatment option with a relatively favorable seizure outcome. These findings suggest that a focal, non-structural and possibly genetic subtype of TLE patients have a relatively high chance of complete removal of the epileptogenic zone, therefore representing good surgical candidacy. Part of the non-lesional TLE patients who do not reach seizure-freedom after resective surgery, may consist of patients with so called 'temporal plus' epilepsy, an electroclinical entity known to have a poor surgical outcome.[18]

Previous studies indicated that mutations in mTOR pathway genes (associated with a structural cause of epilepsy) are an important predictor of good surgical candidacy, especially in patients with no visible lesion on MRI. On the other hand, mutations associated with channel or synaptic transmission function could predict poor surgical candidacy. [6,19]

However, the exact influence of underlying genetic factors on etiological background and associated surgical outcome in non-lesional patients with heterogenous responses to presurgical assessment and surgical treatment remains largely uncharacterized and therefore, it is incompletely known in whom of these patients epilepsy surgery is indicated or contraindicated. In our cohort with surgical patients included between 2000 to 2012, genetic testing was only rarely performed.

The MRI-lesional group in our study had surgical outcomes that were comparable to the lesional group of patients, as based on histopathology results in the full EEBB cohort.[1] This suggests that in a high fraction of patients from the MRI-lesional cohort, no representative tissue sample was obtained during surgery.

This study has some limitations, part of which are inherent to its retrospective design. Despite a positive response rate from most participating centers across Europe we had to exclude 265 patients with missing or inconclusive MRI data from our analysis (32%). Thus, it is possible that non-response, in combination with incomplete data, may have introduced a selection bias. It may be the case that patients in whom insufficient data could be retrieved were more often lost to follow-up because of a favorable outcome. Seizure outcome rates in the centers with the highest response rates, however, were similar to those in the overall group. Also, because MRI-negative patients with positive histopathological findings could not be systematically included in this cohort, the impact of our findings on presurgical decision-making is limited. Next, the group of non-lesional patients who underwent parietal, occipital, or multilobar surgery, was small which compromises the conclusions drawn from these subgroups. Another limitation of the study includes possible differences in histopathological characterization of brain tissue samples amongst the collaborating centers. As a consequence, the presence of mild malformations of cortical development (mMCD, FCD type I, and malformation of cortical development with oligodendroglial hyperplasia and epilepsy: MOGHE) could have been missed on MRI and on histopathological exam in some patients. Spatial sampling errors may have played a role as well, with only parts of the resected specimen and not the entire resected brain tissue, being examined. This error, however, is inherent to all epilepsy surgery series and bears the small risk of not detecting subtle lesions in the resected brain tissue. In addition, the retrospective design does not allow conclusions on the role of invasive EEG recordings prior to epilepsy surgery, and the lack of available data on non-invasive EEG recordings hampers conclusions on its role in surgery outcome in this study. Almost three quarters of the included patients with likely non-lesional epilepsy underwent intracranial EEG. The MD team's reasons to decide for or against iEEG could not be retrospectively assessed but may have differed between centers and influenced patient selection and outcomes. Regarding completeness of resection, information on (expected) completeness of resection as documented by either ACoG or preresection invasive EEG findings was limited to 'complete, 'incomplete, or 'unknown'. Exact reasons for incompleteness of resection were not provided, but in line with general practice we assume that this was either assessed as such when eloquent areas prevented extension of resection, or surgical risks (otherwise) prevented completeness of resection, or the identified area to be epileptogenic (based on pre-resection invasive EEG monitoring) was found to not be completely resected based on post-resection MRI. Lastly, the application of genetic testing is most likely undervalued because of the inclusion period.

With this study, we demonstrate the relatively poor seizure outcomes of MRI-negative focal epilepsy patients who undergo surgery, but in whom no pathological lesional diagnosis can be made. There is an urgent need for expanding the knowledge of pathological substrates and molecular mechanisms underlying non-lesional focal epilepsy and for a long time, genetic analysis only found limited application in unraveling its background. To improve patient selection and surgery outcomes, we need new (possibly genetic) biomarkers for poor surgical candidacy. Patients such as those from this cohort will be a promising group for advanced molecular-genetic analysis of brain tissue specimens in order to identify new brain somatic epilepsy gene mutations or epilepsy-associated molecular pathways.

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Supplemental information

Supplementary table 1. Overview of numbers of enrolled subjects in each participating center.

CENTER	ELIGIBLE CASES, N	COMPLETE CASES, N	LIKELY NON-LESIONAL CASES, N
Barcelona	1	1	1
Belgrado	2	2	0
Bielefeld	27	23	2
Bonn	140	104	21
Erlangen	22	17	11
Florence	9	1	1
Freiburg	14	14	2
Gèneve[a]	50	0	0
Istanbul	12	11	1
KCHL	31	30	18
Kuopio	39	31	18
Leuven	4	4	4
Linz	19	15	3
Lissabon	4	3	0
London (GOSH)	40	23	2
Londen (NHNN)	41	40	20
Lyon (adults)	56	41	16
Lyon (pediatric)	5	4	2
Maastricht[a]	8	0	0
Madrid	3	3	2
Marburg	6	6	0
Milaan	6	3	0
München	17	16	4
Parijs (SAHC)	36	36	33
Parijs (Rothschild)	19	19	3
Pozilli	34	31	12
Praag (adults)	21	19	9
Praag (pediatric)	6	6	1
Rome	4	4	4
Utrecht	53	49	23
Vogtareuth	20	2	1
Wenen	4	3	1
Zürich	11	10	2
Zweden[a]	72	0	0
Total	836	571	217

[a] centers in which no data was retrieved because of no response (2 centers) and lack of possibility to reidentify the included patients from the original EEBB study (1 center)

Supplementary table 2. Baseline characteristics included compared to excluded patients.

CHARACTERISTICS \ COHORT	COMPLETE DATA, INCLUDED (n=571)	INCOMPLETE DATA, EXCLUDED (n=265)	P-VALUE OF DIFFERENCE
age onset – yr, median (IQR)	12.00 (5.00-19.00)	7.00 (3.00-15.00)	0.02
age surgery – yr, median (IQR)	29.00 (19.00-40.00)	24.00 (13.00-37.75)	0.01
duration epilepsy – yr, median (IQR)	13.00 (7.00-23.00)	11.00 (6.00-22.00)	0.10
surgery location			0.10[a]
temporal – no. (%)	349 (61)	163 (62)	0.91
frontal – no. (%)	99 (17)	33 (12)	
parietal – no. (%)	16 (3)	16 (6)	
occipital – no. (%)	13 (2)	12 (5)	
multilobar – no. (%)	90 (16)	40 (15)	
surgery type			0.20
resective – no. (%)	502 (87.9)	160 (87.0)	
disconnective/hemispherotomy – no. (%)	65 (12.1)	14 (13.0)	
surgery outcome			
1 yr Engel 1 – no. (%)	342/561 (61.0%)	95/166 (57%)	0.39
2 yr Engel 1 – no. (%)	271/506 (53.6%)	131/243 (53.9%)	0.98
5 yr Engel 1 – no. (%)	170/331 (51.4%)	86/168 (51.2%)	0.97

[a] p-value for group (trend analysis)

Supplementary table 3. Structural cause based on MRI-lesional (excluded) patients.

MRI-LESIONAL COHORT / MRI-ABNORMALITY	N (%)
all	**354 (100)**
clear focal epileptogenic lesion, but not further specified	118 (33.3)
brain infarct	59 (16.7)
primary brain tumor	39 (11.0)
hippocampal sclerosis	33 (9.3)
FCD	29 (8.2)
traumatic lesion	27 (7.6)
inflammatory lesion	17 (4.8)
hemorrhage	11 (3.1)
MCD (other than FCD)	11 (3.1)
cavernoma	10 (2.8)

Supplementary table 4: genetic testing in cohort of 571 patients.

GENETICS		ANALYSIS					RESULTS	
	N	**individual gene**	**gene panel**	**WES/WGS**	**array**	**other**	**causative findings**	**VUS**
non-lesional	217	2	3	7	1	3	0/16	0
lesional	354	4	3	11	1	7	1/26	3[a]

[a] comprises *SMN1, ARX, and KCNT1* variants

Supplementary table 5. Surgery outcome (Engel class I-IV) in non-lesional temporal versus extratemporal cases.

NON-LESIONAL COHORT / SEIZURE OUTCOME	ALL (%) N=217	TEMPORAL (%) N=150	EXTRATEMPORAL (%) N= 67
ENGEL I			
1 YR	47.4	58.1	22.4
2 YR	40.0	47.3	20.9
5 YR	36.3	41.0	16.4
ENGEL II-III			
1 YR	22.6	18.0	32.8
2 YR	24.9	22.0	31.3
5 YR	14.3	13.3	16.4
ENGEL IV			
1 YR	30.0	23.9	44.8
2 YR	35.1	30.7	47.8
5 YR	49.4	45.7	67.2

Supplementary table 6. Surgery outcome likely non-lesional vs. patients with a structural epileptogenic cause on MRI: temporal *versus* extratemporal.

COHORT	N	1 YR (ENGEL I, %)	2 YR (ENGEL I, %)	5 YR (ENGEL I, %)
non-lesional	**217**	**47.4**	**40.0**	**36.3**
temporal	150	58.1	47.3	41.0
extratemporal	67	22.4	20.9	16.4
frontal	40	22.2	22.2	19.4
parietal	8	12.5	12.5	12.5
occipital	3	33.3	0	0
multilobar	12	41.7	41.7	25
MRI-lesional	**354**	**69.7**	**61.6**	**60.4**
temporal	199	67.5	59.0	57.8
extratemporal	155	72.5	66.4	62.2
frontal	59	70.2	48.9	45.8
parietal	8	50	50	50
occipital	10	60	60	40
multilobar	78	77.6	72.4	72.4

8

What determines the timing of epilepsy surgery in children with cortical malformations and low-grade epilepsy-associated tumors?

Maurits W.C.B. Sanders*, Matyáš Ebel*, Aswin Chari,
Merel Fleumer, Anca M. Vasilica, Maria H. Eriksson,
Martin Tisdall, Barbora Splítková, Anezka Belohlavkova
Willem M. Otte, Floor E. Jansen, J Helen Cross
Kees P.J. Braun**, Pavel Kršek**

*These authors contributed equally to this work.

**These authors jointly directed this work.

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ABSTRACT

Objectives

Timely referral and efficient presurgical evaluation are important for optimizing postoperative seizure and developmental outcomes in epilepsy surgery patients. We aimed to identify determinants of time intervals from epilepsy diagnosis to referral to specialized centers and epilepsy surgery in children with malformations of cortical development (MCDs) and low-grade epilepsy-associated tumors (LEATs).

Methods

We performed a retrospective cohort study in 3 European centers, including children with MCD or LEAT who underwent epilepsy surgery between 2010 and 2020. Primary endpoints were (1) time interval between epilepsy diagnosis and referral for presurgical evaluation, (2) duration of presurgical evaluation trajectory, and (3) time from diagnosis to surgery. The secondary endpoint was postoperative seizure outcome. In our primary analysis, we performed a multivariable Cox proportional hazards analysis, and used histopathological diagnosis, age at first seizure, presence of developmental delay, 1st seizure classification, initial EEG and MRI-findings, duration until drug resistance, and family history as covariates.

Results

Of the 507 children included, 259 (51%) had an MCD and 248 (49%) had a LEAT. Earlier drug resistance and the presence of an MR-visible epileptogenic lesion were associated with shorter intervals from diagnosis to referral and surgery, and later onset of seizures was associated with a shorter time to surgery. LEAT was associated with a shorter presurgical evaluation and shorter time to surgery. An older age at surgery, shorter time from diagnosis to surgery, and a histopathological diagnosis of FCDII and LEAT were all independently associated with better postoperative seizure outcome.

Conclusion

Epilepsy surgery was performed earlier in children with MR-visible lesions and early drug resistance, particularly those with LEAT, and shorter duration to epilepsy surgery was associated with better seizure outcome. Many patients were initially considered MRI-negative in the referring center, highlighting the importance of early referral, epilepsy-dedicated MRI protocols, and experienced neuroradiologists. Increasing awareness of timely referral and surgery benefits is crucial for improving outcomes.

Introduction

Focal drug-resistant epilepsy (DRE) in children who undergo epilepsy surgery is most frequently attributed to malformations of cortical development (MCDs), followed by low-grade epilepsy-associated tumors (LEATs).**1** In children with focal DRE, resective or disconnective surgery is the only potentially curative treatment option, provided that the epileptogenic zone is focal, well-delineated, preferably of presumed structural origin, and located outside eloquent areas.[1,2] A large recent surgical cohort study (the European Epilepsy Brain Bank) reported that 50% of patients with focal cortical dysplasia type I (FCD I) or mild MCD (mMCD), 65% of FCD II patients, and 78% of LEAT patients achieved freedom from disabling seizures 2 years following surgery.[3] A longer duration of epilepsy was shown to correlate with a lower chance of reaching seizure-freedom. This correlation emphasizes the need for a timely referral of focal epilepsy patients to evaluate surgical candidacy as well as a time-efficient presurgical work-up, to avert increased morbidity and mortality associated with DRE and to improve quality-of-life, cognitive and seizure outcomes.[4-6] In young children, however, focal lesional epilepsy may present with generalized or non-lateralized seizures and multifocal epileptiform EEG patterns, challenging early consideration of surgical options. In addition, a considerable subgroup of children with MCDs may have a normal MRI, further complicating presurgical evaluation and patient selection.

Previous studies have identified important predictors of a less favorable postoperative seizure outcome, among which are incomplete resection of the epileptogenic lesion and zone, a histopathological diagnosis of FCD I, extratemporal location of FCDs, normal MRI, and longer epilepsy duration.[3,7–11] Limited knowledge exists regarding the clinical factors that influence the timing of referral for presurgical evaluation and epilepsy surgery. We aimed to identify determinants of time intervals from initial epilepsy diagnosis to referral and to epilepsy surgery, and to associate the interval to surgery with postoperative seizure outcome, in a large multicenter cohort of children with MCDs and LEATs.

Methods

Population

We retrospectively identified consecutive children from 3 European centers (UMC Utrecht, the Netherlands (NL), Great Ormond Street Hospital for Children, London, United Kingdom (UK), and Motol University Hospital, Prague, Czech Republic (CZ)), who underwent epilepsy surgery between 2010 and 2020. The inclusion criteria comprised patients aged 18 years or younger at time of surgery, with histopathologically proven diagnoses of FCD I, mMCD, mMCD with oligodendroglial hyperplasia and epilepsy (MOGHE), FCD II, or LEAT, including FCD IIIb. Neuropathological diagnoses were classified according to ILAE guidelines as follows: FCD I referred to isolated lesions with cortical dyslamination; FCD II referred to cortical dyslamination and the presence of dysmorphic neurons (DNs), with (FCD IIb) or without (FCD IIa) balloon cells (BCs). FCD IIIb referred to cortical dyslamination adjacent to a brain tumor, and mMCD referred to surgical cases with blurred gray-white matter boundaries and excess of heterotopic neurons in cortical layer 1 (mMCD I) or in the deep white matter (mMCD II).[12] MOGHE refers to increased oligodendroglia and heterotopic neurons in the white matter.

Endpoints and data collection

Primary endpoints were the time intervals from epilepsy diagnosis to referral for presurgical evaluation, duration of presurgical evaluation, and from diagnosis of epilepsy to surgery (in months). If a patient underwent multiple surgeries, the first surgery was used for the calculation of the time intervals. Surgery data were obtained and included invasive EEG monitoring, type of surgery, reoperation, and surgical outcome at latest follow-up, according to the Engel classification.[13] If a patient underwent more than one surgery, the surgical outcome after the last surgery was considered. Clinical data included: family history of epilepsy (1st or 2nd degree relatives), history of febrile seizures, developmental delay, neurological deficits, as well as the classification and time of first seizures (all of these as documented by the physician at the time of first consultation and neurological examination), the results of initial EEG (categorized according to the presence or absence of focal epileptiform abnormalities) and MRIs (as described in the original reports; the absence of a visible causative lesion was defined as MRI-negative), the treatment regimen (e.g., effectiveness of the first anti-seizure medication [ASM] and total number of ASMs ever used), and development of DRE.[14] The moment of DRE diagnosis was defined as the time of initiation of the third ASM. Alternatively, if no third ASM was initiated, the time of DRE was determined by the lack of achieving seizure-freedom while on the second ASM after a treatment duration of at least three times the baseline seizure interval ('rule of 3').[15]

Statistical analysis

Predictors of seizure outcome, selected based on their identification as outcome determinants in previous studies, were analyzed in relation to the 3 time intervals using a multivariable Cox proportional hazards model with covariates: histopathological diagnosis (FCD I, mMCD, MOGHE, FCD II, or LEAT), family history of epilepsy (positive or negative), age at the first seizure (in months), developmental delay (yes or no at time of first evaluation), 1st seizure classification (focal or non-focal), 1st EEG result (reported as with or without focal epileptiform abnormalities) initial MRI report (negative or epileptogenic lesion identified) and time until DRE (in months).[3,7–11] Continuous variables were transformed into binary variables based on the median as threshold. Missing data were analyzed through multiple imputations using predictive mean matching. Heterogeneity between centers was tested, and when present, random-effects modeling was used.

As a secondary analysis, we performed a multivariable logistic regression analysis to calculate odd ratios, converted to (adjusted) risk ratios, in patients who became seizure-free compared to those with recurrent seizures after surgery, associated with the following available and possibly relevant determinants of seizure outcome that we used as covariates: age at surgery, duration from diagnosis to resective surgery (in months), MRI-findings, and histopathological diagnosis.

We applied a False Discovery Rate (FDR) correction to all variables with a significant p-value in the primary analysis to address the potential impact of multiple testing. Data analysis was performed with IBM SPSS 23 and R Statistical software version 4.3.2. The medical ethics committees of the 3 participating centers approved the study and concluded that informed consent was not required for this retrospective observational study on available anonymized data from routine clinical care.

Results

Patient characteristics

We included data from 507 patients (176 from NL, 155 from CZ, and 176 from the UK), of whom 49 (10%) had FCD I, 11 (2%) mMCD, 4 (1%) MOGHE, 195 (38%) FCD II, and 248 (49%) –including 32 FCD IIIb patients– LEAT. Clinical characteristics are summarized in **table 1**. FCD II patients had a relatively longer median duration (in months) from epilepsy diagnosis to referral (38, IQR 12–88) and to surgery (61, IQR 26–105), whereas FCD I, mMCD, and MOGHE patients had a relatively longer duration of presurgical evaluation (12, IQR 6-20). FCD II patients (66; 34%) relatively more often had a positive family history. Nearly all (98%) LEAT patients had a suspected epileptogenic lesion reported on the initial MRI at the referring center, compared to 34 (63%) of FCD I and 138 (76%) of FCD II patients. These numbers increased to 43 (76%) of FCD I and 179 (96%) of FCD II patients following MR imaging and review in the centers where presurgical evaluation was performed. In 45 out of 70 (64%) patients initially considered MRI-negative at the referring centers, a causative lesion was successfully identified at the tertiary epilepsy surgery centers. Almost all FCD patients were diagnosed with DRE before surgery, compared to 137 (75%) of LEAT patients. LEAT patients (12; 6%) seldom underwent invasive EEG-monitoring during the presurgical evaluation. Early onset of DRE (within 16 months after medication initiation) was seen in less than half of the FCD II and LEAT patients, in contrast to almost three-quarters of patients diagnosed with FCD I, mMCD, or MOGHE. There were 316 patients (78%) with focal epileptiform abnormalities at the first EEG, 65 patients (16%) had a normal EEG, and 26 patients (6%) had generalized or bilateral EEG abnormalities.

Table 1. Baseline characteristics of patients (N=507).

CHARACTERISTICS	COHORT	FCD1, mMCD, or MOGHE N=64	FCDII N=195	LEAT N=248	ALL N=507
time intervals	epilepsy diagnosis to referral – months (mo.) median (IQR)	26 (4–39)	38 (12–88)	17 (5–53)	25 (6–65)
	duration presurgical evaluation – mo. median (IQR)	12 (6–20)	8 (3–18)	6 (3–13)	7 (3–15)
	epilepsy diagnosis to surgery – mo. median (IQR)	41 (24–65)	61 (26–105)	29 (12–66)	40 (18–86)
patient demographics	male sex – no. (%)	28 (43)	107 (55)	111 (45)	246 (49)
	family history of epilepsy – no. (%)	7 (11)	66 (34)	55 (22)	128 (25)
first seizure	age at first seizure – mo. median (IQR)	27 (5–73)	25 (6–51)	65 (15–120)	36 (10–89)
	focal classification – no. (%)[a]	45 (76)	156 (82)	201 (83)	402 (82)
	body temperature > 38.5 °C – no. (%)	2 (3)	14 (7)	5 (2)	21 (4)
initial evaluation	age at diagnosis – mo. median (IQR)	37 (8–81)	35 (9–70)	66 (16–131)	40 (12–96)
	neurological deficit – no. (%)[b]	3 (5)	24 (13)	5 (2)	32 (7)
	developmental delay– no. (%)[c]	13 (24)	48 (28)	59 (28)	120 (27)
	focal epileptiform abnormalities at first EEG[d] – no. (%)	30 (71)	128 (80)	159 (78)	316 (78)
	suspected epileptogenic lesion reported on first MRI[e] – no. (%)	34 (63)	138 (76)	230 (98)	401 (85)
course of epilepsy	seizure-free (Engel 1) after 1st ASM– no. (%)	13 (20)	70 (36)	50 (20)	133 (26)
	DRE – no. (%)[f]	45 (97)	146 (94)	137 (75)	328 (83)
	duration until DRE – mo. median (IQR)	11 (6–17)	19 (6–53)	17 (6–47)	16 (6–41)
	number of ASM > 5 – no. (%)[g]	26 (53)	100 (63)	18 (9)	144 (36)
epilepsy surgery	suspected epileptogenic lesion reported on final MRI[e] – no. (%)	41 (76)	175 (96)	235 (100)	451 (95)
	invasive EEG – no. (%)[h]	13 (27)	43 (27)	12 (6)	68 (17)
	age at surgery – mo. median (IQR)	104 (42–172)	106 (61–163)	125 (65–177)	114 (58–173)

CHARACTERISTICS	COHORT	FCDI, mMCD, or MOGHE N=64	FCDII N=195	LEAT N=248	ALL N=507
	seizure-free (Engel I) at last follow-up – no. (%)[i]	35 (58)	154 (79)	208 (84)	394 (78)
	duration of follow-up – mo. median (IQR)	30 (15–60)	27 (15-45)	30 (18–50)	30 (18–47)

[a] no definitive data on seizure classification could be retrieved in 5 (8%) FCD1/mMCD,/MOGHE patients, 5 (3%) FCD II patients, and 9 (4%) LEAT patients
[b] no definitive data on first neurological exam could be retrieved in 5 (8%) FCD1/mMCD,/MOGHE patients, 10 (5%) FCD II patients, and 9 (4%)LEAT patients
[c] no definitive data on developmental delay could be retrieved in 11 (17%) FCD1/mMCD,/MOGHE patients, 24 (12%) FCD II patients, and 32 (13%) LEAT patients
[d] no definitive data on first EEG findings could be retrieved in 22 (34%) FCD1/mMCD,/MOGHE patients, 34 (17%) FCD II patients, and 44 (1 8%) LEAT patients
[e] no definitive data on first MRI findings could be retrieved in 10 (16%) FCD1/mMCD,/MOGHE patients, 13 (7%) FCD II patients, and 13 (5%) LEAT patients
[f] no definitive data on DRE diagnosis could be retrieved in 17 (27%) FCD1/mMCD/MOGHE patients, 40 (21%) FCD II patients, and 53 (21%) LEAT patients
[g] no definitive data on total number of ASM could be retrieved in 15 (23%) FCD1/mMCD/MOGHE patients, 37 (19%) FCD II patients, and 53 (21%) LEAT patients
[h] no definitive data on total invasive diagnostics could be retrieved in 15 (23%) FCD1/mMCD/MOGHE patients, 36 (18%) FCD II patients, and 53 (21%) LEAT patients
[i] no definitive data on seizure outcome following epilepsy surgery could be retrieved in 4 (6%) FCD1/mMCD/MOGHE patients, and 1 (0.4%) LEAT patient.

Primary analysis

Multivariable Cox proportional hazard analysis results are listed in **table 2**: a hazard ratio (HR) > 1 indicates a higher likelihood of a shorter time interval than the reference; missing data was imputed (**table 1**).

A shorter interval from epilepsy diagnosis to referral for presurgical evaluation was associated with early presence of DRE (<15 months; HR 2.47; 95% CI 1.97–3.11, p-value <0.01), focal epileptiform abnormalities on the first EEG (HR 1.55; 95% CI 1.09–2.20, p-value=0.02), and the presence of an MRI-visible epileptogenic lesion (HR 1.43; 95% CI 1.03–2.00, p-value=0.03).A shorter duration of presurgical evaluation was associated with the histopathological diagnosis LEAT (HR 1.71; 95% CI 1.26–2.34, p-value <0.01). A shorter interval from epilepsy diagnosis to epilepsy surgery was associated with early onset of DRE (< 16 months; HR 2.37; 95% CI 1.90–2.78, p-value <0.01), the presence of a lesion on first MRI (HR 1.44; 95% CI 1.03–1.68, p-value=0.03), a histopathological diagnosis of LEAT (HR 1.41; 95% CI 1.04–1.58, p-value=0.04), and later age at onset of seizures (> 36 months; HR 1.28; 95% CI 1.04–1.58, p-value=0.03). All reported p-values remained below the 0.05 significance threshold following FDR correction.

Our analysis did not show significant associations between the time intervals and the first seizure classification, family history of epilepsy, or the presence of developmental delay at the time of epilepsy diagnosis.

Table 2. Outcomes of multivariate Cox proportional hazards analysis for the 3 primary endpoints (time intervals): a hazard ratio > 1 implies a higher chance of shorter duration compared to the reference.

TIME / INTERVAL N=507	1: DIAGNOSIS TO REFERRAL		2: DURATION EVALUATION		3: DIAGNOSIS TO SURGERY	
	hazard ratio (95% CI)	p-value	hazard ratio (95% CI)	p-value	hazard ratio (95% CI)	p-value
etiology						
FCDI/mMCD/MOGHE	reference		reference		references	
FCDII	0.74 (0.54-1.03)	0.07	1.28 (0.94-1.73)	0.12	0.85 (0.48-1.15)	0.31
LEAT	1.06 (0.76-1.49)	0.72	1.71 (1.26-2.34)	<0.01	1.41 (1.04-2.00)	0.04
age at 1st seizure						
< 36 months	reference		reference		Reference	
≥ 36 months	1.19 (0.96-1.47)	0.11	1.06 (0.88-1.28)	0.55	1.28 (1.04-1.58)	0.02
developmental delay						
no	reference		reference		reference	
yes	0.96 (0.76-1.23)	0.77	1.02 (0.82-1.27)	0.97	0.98 (0.76-1.26)	0.88
1st seizure classification						
non-focal	reference		reference		reference	
focal	1.03 (0.77-1.28)	0.95	1.03 (0.82-1.30)	0.79	1.10 (0.86-1.42)	0.45
1st EEG focal abnormalities						
no	reference		reference		reference	
yes	1.55 (1.09-2.20)	0.02	0.80 (0.60-1.07)	0.14	1.30 (0.92-1.84)	0.14
1st MRI outcome						
negative	reference		reference		reference	
positive	1.43 (1.03-2.00)	0.03	1.23 (0.92-1.58)	0.17	1.44 (1.03-1.68)	0.03
duration until DRE[a]						
≥ median[b]	reference		reference		reference	

TIME / INTERVAL N=507	1: DIAGNOSIS TO REFERRAL		2: DURATION EVALUATION		3: DIAGNOSIS TO SURGERY	
	hazard ratio (95% CI)	p-value	hazard ratio (95% CI)	p-value	hazard ratio (95% CI)	p-value
< median[b]	2.47 (1.97-3.11)	<0.01	1.20 (0.97-1.49)	0.10	2.37 (1.90-2.78)	<0.01
family history of epilepsy						
negative	reference		reference		reference	
positive	0.94 (0.75-1.17)	0.59	- [c]	-	- [c]	-

[a] patients who developed DRE after the start of the presurgical evaluation were considered as not drug-resistant for this endpoint
[b] the median age of duration until DRE was 15 months for patients who developed DRE before the start of the presurgical evaluation, and 16 months for the entire cohort
[c] family history of epilepsy was included in the analysis solely for the time interval from diagnosis to referral

Secondary analysis

Postoperative seizure outcome data were available in 502 of 507 (99%) cases. On average, 78% of all patients reached seizure-freedom (Engel 1) at the latest follow-up. Seizure-freedom ranged from 58% in FCD I, mMCD, and MOGHE cases, to 79% in FCD II cases, and 84% in LEAT cases. The median follow-up durations for these groups were 30, 27, and 30 months, respectively.
Multivariable analysis (**table 3**) showed a significant association between seizure-freedom at last follow-up and the following determinants: an older age at surgery (adjusted risk-ratio (ARR) per 3 months: 1.02; 95% CI, 1.01-1.03), a shorter interval from epilepsy diagnosis to surgery (ARR per 3 months: 0.96; 95% CI, 0.96-0.97), and a histopathological diagnosis of FCD II (ARR: 1.74; 95% CI, 1.25-2.43) and LEAT (ARR: 2.47; 95% CI, 1.61-3.78).

Table 3. Multivariable regression analysis in all patients with favorable (Engel I) *versus* unfavorable outcome (Engel II–IV) following surgery.

SEIZURE OUTCOME / CHARACTERISTICS	ENGEL I[a] n=394	ENGEL II-IV[a] n=108	MULTIVARIABLE ADJUSTED RISK RATIO (95% CI)[b]	P-VALUE
age surgery – mo. median (IQR)	98 (44–158)	87 (41–134)	1.02 (1.01–1.03)[c]	<0.01
time from diagnosis to surgery – mo. median (IQR)	14.5 (8.1–21.5)	19 (9.0–26.75)	0.96 (0.96–0.97)[c]	<0.01
lesion on 1st MRI – no. (%)	62 (82)	69 (60)	0.76 (0.36–1.50)	0.60
etiology				
FCDI/mmCD/MOGHE – no. (%)	35 (9)	25 (23)	reference	
FCDII – no. (%)	154 (39)	42 (39)	1.74 (1.25–2.43)	<0.01
LEAT – no. (%)	208 (53)	41 (38)	2.47 (1.61–3.78)	<0.01

[a] Engel classification at last FU years follow-up, 5 (1%) missing cases (4 FCD 1/mMCD/MOGHE, 1 LEAT)
[b] adjusted for all other covariates
[c] risks ratios per 3 months

Discussion

We studied a cohort of 507 European pediatric epilepsy surgery patients diagnosed with FCD I, mMCD, MOGHE, FCD II or LEAT to analyze clinical factors influencing the time interval from initial epilepsy diagnosis to referral, presurgical evaluation duration, and time interval from diagnosis to epilepsy surgery. Earlier drug-resistant epilepsy (DRE), focal epileptiform abnormalities on the initial EEG, and later seizure onset were independently associated with a shorter time from diagnosis to referral. A histopathological diagnosis of LEAT was the only determinant of a shorter presurgical evaluation duration. Earlier presence of DRE, the presence of a lesion on the first MRI, a histopathological diagnosis of LEAT, and later onset of seizures were associated with a shorter time interval to surgery. Seizure outcome was better in patients with a shorter time to surgery, and in FCD II and LEAT patients.

Previous studies indicated that despite recent improvements in early referral for surgical evaluation in pediatric epilepsy, the average duration of epilepsy before surgery remains substantial, recently reported as 5.3 years.[1] Delay to surgery often stems from late referral for surgical evaluation, which is likely influenced by concerns regarding surgery-associated risks, particularly in young children, combined with incomplete understanding of the potential benefits of surgery and expanding indications.[16,17] Challenges in accurately identifying surgical candidates also include the frequent finding of generalized or bilateral EEG abnormalities in young children that may mask

a focal structural etiology, and the failure of standard MR imaging techniques to detect (micro) structural lesions.[18]

In alignment with these findings, our study demonstrates that more than one out of five patients lacked focal epileptiform abnormalities as reported on their initial EEG, significantly delaying referral for presurgical evaluation. Moreover, in almost two-thirds of initially MRI-negative patients, primarily those with FCD, a lesion was detected on subsequent MR imaging and evaluation during the presurgical evaluation in expert hands. The initial under-detection of these lesions also contributes to the delays in referral and surgery, given the historical perception of MRI-negative patients representing lower surgical candidacy. LEAT patients had shorter evaluation times, which is likely due to concordance of presurgical investigations and to MR-visibility of the lesion, requiring fewer additional diagnostics nor invasive recordings prior to resective surgery. The finding that earlier seizure onset is independently related to a longer interval to surgery may be partly attributed to concerns about surgical risks, particularly in younger children who often require relatively extensive resections. Unsurprisingly, DRE was associated with earlier referral and surgery.

Consistent with previous reports,[3,7–10] our study demonstrates that seizure outcomes following surgery are most favorable in LEAT patients and least in the FCD I, mMCD, or MOGHE group. Furthermore, we observed that for every 3-month increase in age at surgery – adjusted for MRI-findings, delay to surgery, and for histopathological diagnosis– the chance of achieving seizure-freedom increased by 2%. It has been suggested that younger age at surgery and earlier seizure onset indicate the presence of more extensive epileptic networks, challenging completeness of resection.[19] Importantly, for every 3-month increase in duration from epilepsy diagnosis to surgery, the chance of achieving seizure-freedom after surgery decreased by 4%. These results align with prior observational cohorts reporting that patients with shorter epilepsy duration are more likely to achieve seizure-freedom,[3,11] suggesting that prolonged exposure to epileptic seizures may lead to more complex or widespread epileptogenic networks. Additionally, a shorter duration of epilepsy and ASM exposure is likely to improve cognitive functioning, especially in the developing child.[6,20,21]

There are several limitations inherent to a retrospective analysis. We could not retrieve complete data for all variables used in our primary analysis. Consequently, we addressed this issue by imputing missing data. Regarding our secondary endpoint, we minimized potential confounding bias by including known predictors of postoperative outcome in our analysis. Also, there is an underestimation of the histopathological entity MOGHE in our study, as, at the time of surgery for most patients in our cohort, MOGHE was not yet widely recognized as a distinct pathological entity.

In conclusion, epilepsy surgery was performed earlier, as expected, in children with visible lesions on initial MRI and with early drug resistance, with those having LEAT requiring shorter evaluation times. Longer epilepsy duration relates to poorer outcome. Historically, MRI-negative patients are considered less eligible for surgery, but many have focal epileptogenic brain lesions undetectable on initial MRI in non-specialized centers. This highlights the need for early referral, use of epilepsy-dedicated diagnostic protocols, and experienced neuroradiologists. Increasing awareness of the benefits of timely referral and surgery, which might be facilitated by referral prior to fulfilling the traditional definition of DRE, is crucial for improving postoperative seizure outcomes, especially in children with suspected lesional focal epilepsy despite a normal MRI.

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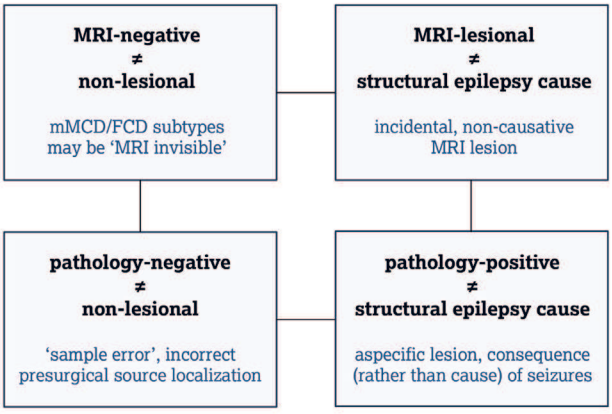
General discussion

The aim of this thesis was to assess the clinical importance of genetic diagnostics in patients, particularly children, evaluated for drug-resistant focal epilepsy as part of the presurgical work-up. While the identification of a structural lesion on MRI remains the classical gateway to epilepsy surgery,[1-4] an increasing number of children are now being considered for surgery even in the absence of a causative, MRI-visible lesion.[5-10] Over the past decades, genetic research has reshaped the conceptual framework of epilepsy, revealing that focal epilepsies may arise from a wide range of germline and somatic variants, with or without visible structural correlates.[11-14] The studies presented in this thesis collectively demonstrate that genetic diagnostics provide etiological, prognostic, and therapeutic insights into the context of epilepsy surgery, improving patient selection, timing of intervention, and expected surgical outcomes.

Pitfalls in the presurgical work-up

Understanding the limitations of current presurgical approaches is essential to identify opportunities for further improvement of patient selection and surgery outcome. The primary objective of the presurgical evaluation is to accurately delineate the epileptogenic zone that must be resected to achieve seizure-freedom.[15,16] This task remains challenging, particularly in MRI-negative patients or in cases where histopathology yields inconclusive results, possibly hindering a timely and targeted treatment.[16,17] High-resolution MRI plays a pivotal role in source localization,[4,16,17] yet certain entities such as mild malformations of cortical development (mMCD) or subtle focal cortical dysplasias (FCD) may remain invisible on standard imaging; an MRI-negative patient is not necessarily non-lesional.[10,18] Conversely, MRI abnormalities may be incidental, occurring in up to 5% of patients, and their presence may not causally be related to seizures.[20,21] Furthermore, histopathological analysis can yield false-negative results due to sampling error or inaccurate presurgical localization.[10,21,22] In contrast, pathology may also reveal non-specific or incidental lesions that are not causally related to the epilepsy or may reflect secondary rather than causative abnormalities.[23-25] These potential pitfalls in presurgical source localization are illustrated in **figure 1**.

Figure 1. Pitfalls in the presurgical work-up.

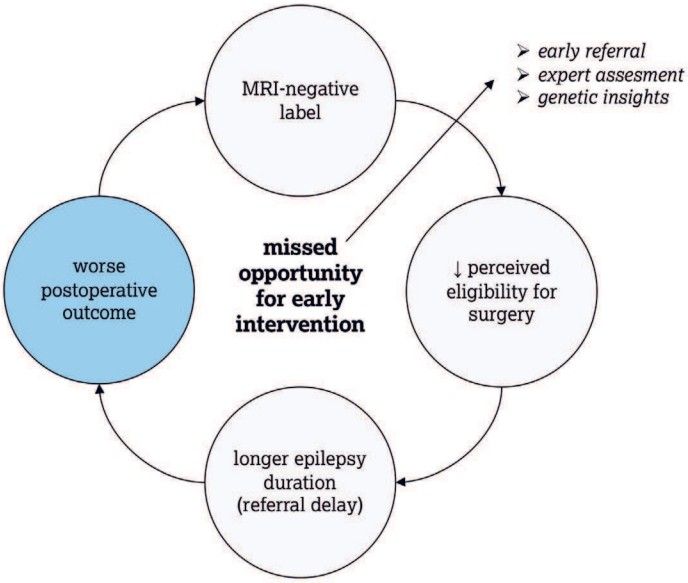


The MRI-negative paradox

Historically, the absence of visible lesions on MRI has been associated with poorer surgical outcomes.[4-7] This perception stems from the notion that MRI-negativity reflects a diffuse or non-lesional form of epilepsy.[9,16,17] However, the label 'MRI-negative' may also trigger a series of decisions that cumulatively disadvantage the patient.[26-28] This may give rise to a self-reinforcing bias, in which delayed or withheld evaluation for surgery contributes to poorer outcomes and strengthens the belief that MRI-negative patients are inherently less eligible for surgery.

Figure 2 illustrates this self-reinforcing loop: MRI-negative patients are perceived as less eligible for surgery, leading to delayed referral and prolonged presurgical investigations. Consequently, the interval during which patients continue to have seizures becomes longer, and this delay itself is independently associated with lower rates of postoperative seizure-freedom and negative impact on cognitive outcomes. The result is a retrospective confirmation of the initial doubt: 'MRI-negative' equals 'poor prognosis', reinforcing future clinical caution when considering MRI-negative patients for epilepsy surgery. Considering three findings supported by our data, this pattern is particularly problematic. First, MRI-negativity is often a fluid rather than fixed category. Upon re-evaluation in expert centers, a substantial proportion of patients initially labeled as MRI-negative were found to have identifiable, causative lesions. [15-17,21] Second, even in truly MRI-negative patients, favorable surgical outcomes can still be achieved, particularly when the presurgical evaluation trajectory, including genetic analysis, points to a focal, structural cause of epilepsy.[7-10, 29-31] Importantly, MRI-negativity does not preclude the presence of a lesion that is detectable on histopathological examination. [6,7,10]

Figure 2. The MRI-negative paradox.



Third, the predictive value of MRI-negativity appears to be mediated, at least in part, by its impact on timing. Poorer outcomes associated with MRI-negativity may therefore reflect both the intrinsic characteristics of the underlying epileptogenic network itself and delayed intervention. Early molecular evidence can help to break this cycle by guiding targeted further work-up, avoiding unnecessary invasive diagnostics, and accelerating decision-making.[21,27,28]

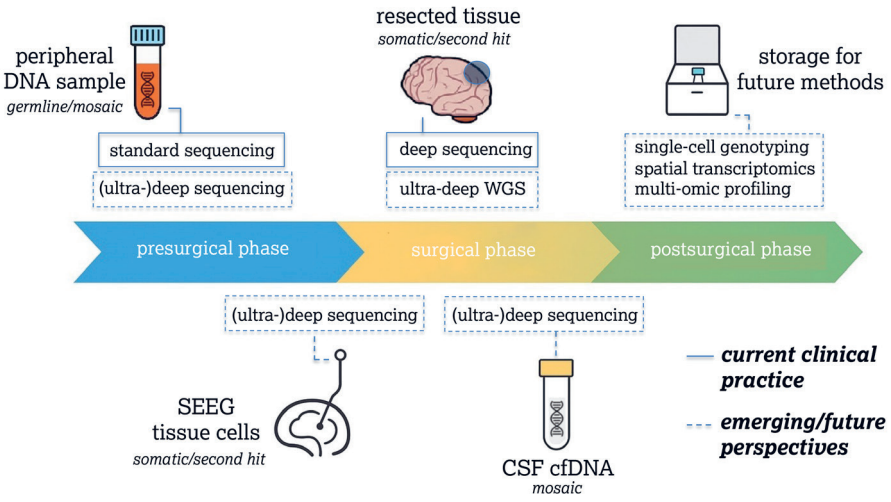
Genetics in epilepsy: the road to cure

A decade ago, genetic testing played only a limited role in proposed diagnostic algorithms, mostly in cases of focal cortical dysplasia for whom the conventional assessment was of uncertain value for predicting surgical candidacy.[32] Since then, the landscape has shifted. Cumulative evidence, including our own findings, indicate that, when integrated with clinical phenotyping, genetic testing can make meaningful contributions to preoperative decision-making. Early germline analysis can help distinguish focal epilepsy patients with diffuse pathophysiological mechanisms (where resective surgery is unlikely to achieve seizure-freedom) from those with a focal structural cause.[29-31,36] Importantly, our findings show that such testing has often been performed too late (or not at all), which, in the clinical trajectory, effectively represents a missed opportunity not only to optimize patient selection but also to avoid unnecessary invasive interventions. It also underscores a missed opportunity to provide timely and accurate counselling to patients and their families, for example regarding surgical prognosis and considerations related to familial epilepsy.

Figure 3 outlines a linear framework for integrating genetics into the surgical pathway, structured across three phases. This framework should be considered consistently and early in all surgical candidates. In the presurgical phase, germline testing from peripheral DNA, ideally initiated as soon as drug resistance is suspected (or even earlier), could identify pathogenic variants that provide molecular evidence for a focal, structural cause of epilepsy, regardless of MRI-findings. For MRI-negative patients, early genetic confirmation of a focal etiology strengthens the case for expedited referral and comprehensive evaluation in an expert center. This approach aligns with the principle that earlier surgical intervention is associated with better seizure and cognitive outcomes, supporting the concept of a biological window of opportunity.[27,28] Prospects in this stage include the application of ultra-deep sequencing methods with error correction, which enables one to detect (ultra-)low-level mosaicism well below the sensitivity of standard methods. By increasing the diagnostic yield, these approaches may further improve precise and timely surgical decision-making.

In the surgical phase, somatic variant analysis of resected tissue substantially increases molecular diagnostic yield. High-depth sequencing can detect low-level mosaic variants in mTOR-pathway genes and other epileptogenic mechanisms, many below the threshold of conventional methods.[35-37] A key practical advance, specific to mTOR-related lesions, is cell-state–guided enrichment. Fluorescence-activated sorting of p-S6–positive cells, followed by deep whole-genome sequencing, can reveal ultra-low-level variants (down to <0.2% VAF) that would otherwise remain undetectable.[38] When combined with structural-variant analysis, this approach has raised molecular detection rates to around 80% in FCD II cohorts.[39]

Figure 3. Genetic testing in epilepsy surgery.



In some germline-positive cases, a second somatic hit can be identified, supporting a two-hit model of pathogenesis, partly explaining phenotypic gradients within the same genetic background.[40] These findings not only elucidate the underlying pathophysiological mechanisms, particularly in cases with inconclusive MRI or histopathology, but also clarify prognostic expectations, as certain variants are associated with distinctly different postoperative seizure outcomes. Moreover, they inform genetic counselling and may also provide a foundation for the development of targeted therapies and improved presurgical strategies in future patients. Another emerging perspective is S-EEG-guided sampling, using electrode-adherent cells or micro-aspirates, to enable targeted detection of somatic variants during presurgical evaluation, which may be particularly valuable in patients who ultimately do not undergo resective epilepsy surgery and in whom genetic testing of blood DNA remains unrevealing.[41] Analysis of cerebrospinal fluid cell-free DNA obtained intraoperatively can capture mosaic variants even when resected tissue is limited or histopathology is inconclusive.[42]

Postoperatively, ultra-deep sequencing technologies, together with single-cell, spatial, and multi-omic profiling, could improve the detection of ultra-low-frequency variants and their spatial mapping within the epileptogenic zone.[43] Such integrated approaches may advance clinico-pathological–genomic classification of focal cortical malformations, guiding both prognosis and expand targeted therapeutic options.

The implementation of genetic testing as a staged model with early germline testing, systematic somatic tissue analysis, and adoption of minimally invasive or ultra-sensitive methods can resolve surgical candidacy, especially in challenging MRI-negative cases, enhance patient counselling, and align treatment timing with the biological window of opportunity for successful surgery.

Non-lesional epilepsy: unresolved questions

The main limitation of this thesis is the incomplete characterisation of patients with non-lesional focal epilepsy. We found that 41% of temporal and 16% of extratemporal non-lesional patients achieved long-term seizure-freedom postsurgically,[10] which underscores the heterogeneity of clinical outcomes in this cohort. While germline variants such as *DEPDC5* or somatic variants such as *SLC35A2* can be expected in a subset of cases,[35] the molecular mechanisms underlying non-lesional focal epilepsy remain incompletely understood. Precisely this lack of insight, together with the variability in postoperative outcomes, illustrates the shortcomings of current presurgical decision-making and patient selection in non-lesional epilepsy. Reliable determinants to predict surgical candidacy in this group are lacking, particularly in extratemporal cases where surgical outcomes are generally poor. Future studies should therefore prioritize the integration of novel, in-depth molecular-genetic approaches with presurgical assessment and histopathological characterization. This will help to identify new biomarkers that can distinguish eligible from non-eligible candidates, predict outcome, and extend the current genetic knowledge of focal non-lesional epilepsies.

Main conclusions

1. Epilepsy surgery can be successful in patients with an established genetic cause, but outcomes vary depending on the underlying pathophysiological mechanism (Chapter 3).
2. Genetic testing can influence clinical decision-making and should be considered consistently and at an early stage in all patients who may become surgical candidates (Chapters 3 and 4).
3. Patients with GATOR1-related familial FCD represent a challenging subgroup with a phenotypic gradient across generations and poorer subsequent surgical outcomes (Chapter 5).
4. In some non-lesional patients, low-level somatic variants in tissue and mosaicism in blood were detected (Chapter 6).
5. The true prevalence of somatic variants in mild malformations of cortical development and non-lesional cases is likely underestimated, underscoring the need for highly sensitive sequencing approaches to improve diagnostic yield and clinical decision-making (Chapter 6).
6. Even so-called non-lesional patients, in whom no causative lesions are detected on MRI and in histopathology and of whom the information on underlying genetic causes is incomplete, particularly those undergoing mesiotemporal resections, may achieve long-term seizure-freedom (Chapter 7).
7. The low rate of successful surgery in extratemporal non-lesional patients highlights the need for in-depth molecular characterization, including tissue-based genetic testing, in order to identify novel predictive markers of surgical candidacy (Chapter 7).
8. A shorter time from epilepsy diagnosis to surgery is independently associated with higher rates of postoperative seizure-freedom (Chapter 8).
9. The absence of clear MRI findings should prioritize early expert-center presurgical evaluation (Chapter 8).

Epilepsy surgery remains the only potentially curative treatment for patients with focal drug-resistant epilepsy, and even those whose seizures are well controlled with medication almost always remain medication-dependent. The findings presented in this thesis support a presurgical pathway in which genetic insights are integrated early and consistently, alongside reliable application of established prognostic factors and timely referral to expert centers. Such an approach will reduce diagnostic delays, optimize patient selection, and ultimately improve long-term outcomes.

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Appendix

Authors and affiliations

Aichholzer, Martin[1,2]
Amorim, Isabel[3]
Aronica, Eleonora[4,5]
Arzimanoglou, Alexis[6,7]
Baldassari, Sara[8]
Barba, Carmen[9]
Becker, Albert[10]
Beckervordersandforth, Jan[11]
Belohlavkova, Anezka[12]
Bien, Christian G.[14]
Blümcke, Ingmar[15]
Bodi, Istvan[16]
Braun, Kees P.J.[17]
Brilstra, Eva H.[18]
Buccoliero, Anna Maria[19]
Catenoix, Helene[20,21]
Chari, Aswin[22]
Chassoux, Francine[23]
Chipaux, Mathilde[24]
Cloppenborg, Thomas[14]
Coras, Roland[15]
Cross, J. Helen[22,27]
De Palma, Luca[63]
De Tisi, Jane[26]
Deleo, Francesco[53]
Devaux, Bertrand[23]
Di Gennaro, Giancarlo[30]
Dorfmüller, Georg[32]
Duncan, John S.[26]
Ebel, Matyas[12]
Eriksson, Maria H.[22]
Esposito, Vincenzo[30,31]
Feucht, Martha[54]
Fleumer, Merel[17]
Garbelli, Rita[53]
Giordano, Flavio[46,47]
Gil-Nagel, Antonio[56]
Grunwald, Thomas[45]
Grote, Alexander[21,50]
Guerrini, Renzo[9]
Hamer, Hajo[15]
Hartlieb, Till[40,41]
Helmstaedter, Christoph[34]
Hermanovska, Barbora[12]
Honavar, Mrinalini[16]
Jacques, Thomas S.[26]
Jansen, Floor E.[17]
Kälviäinen, Reetta[28]
Kalina, Adam[39]
Kang, Hoon Chul[35]
Kaufmann, Elisabeth[37]
Ko, Ara[35]
Koenig, Kristina[45]
Koeleman, Bobby P.C.[18]
Krsek, Pavel[12,39]
Kudernatsch, Manfred[40,41]
Lal, Dennis[62]
Lee, Jeong Ho[36]
Lemmens, Cynthia M.C.[17]
Marusic, Petr[39]
Menzler, Katja[49]
Noachtar, Soheyl[37]
Novak, Vilem[33]
Otte, Willem M.[17]
Özkara, Cigdem[44]
Pimentel, Jose[3]
Pieper, Tom[40]
Rácz, Attila[34]
Raicevic, Savo[57]
Rauramaa, Tuomas[28,29]
Rheims, Sylvain[20,21]
Ristic, Aleksandar[58,59]
Rössler, Karl[55]
Rosenow, Felix[48,49]
Rumiá, Jordi[61]
San Antonio-Arce, Victoria[61]
Sanders, Maurits W.C.B.[17]
Scheiwe, Christian[42,43]
Schijns, Olaf[60]
Scholl, Theresa[54]
Schulze-Bonhage, Andreas[42]
Sim, Nam Suk[36]
Specchio, Nicola[63]
Splitkova, Barbora[12]
Stanek, David[13]
Stevelink, Remi[17]
Straka, Barbora[39]
Surges, Rainer[34]
Theys, Tom[52]
Tisdall, Martin[22]
Toledano Delgado, Rafael[56]
Toulouse, Joseph[6]
Tuinman, Maarten P.[17]
Uhrova-Meszarosova, Anna[12,13]
Van der Wolf, Iskander J.[17]
Van Paesschen, Wim[51]
Vasilica, Anca M.[22]
Vlckova, Marketa[39]
von Oertzen, Tim J.[1,2]
Zamecnik, Josef[39]

1. Johannes Kepler University, Faculty of Medicine, Linz, Austria
2. Kepler University Hospital, Department of Neurosurgery, Linz, Austria
3. Hospital de Santa Maria (CHULN), Laboratory of Neuropathology, Lisbon, Portugal
4. Department of Neuropathology, Amsterdam UMC, Amsterdam, the Netherlands
5. Stichting Epilepsie Instellingen Nederland (SEIN), Heemstede, the Netherlands
6. University Hospitals of Lyon (HCL), Dept. of Pediatric Clinical Epileptology, Lyon, France
7. Paediatric Epilepsy Unit, Child Neurology & Neurosurgery, Hospital Sant Joan de Déu, Barcelona, Spain
8. Institut du Cerveau – Paris Brain Institute, Sorbonne Université, Paris, France

9. Meyer Children's Hospital, Neuroscience Department, Florence, Italy
10. Department of Neuropathology, University of Bonn Medical Center, Bonn, Germany
11. Maastricht University Medical Center, Department of Pathology, Maastricht, Netherlands
12. Department of Paediatric Neurology, Motol Epilepsy Center, Prague, Czech Republic
13. Neurogenetics Laboratory, Department of Paediatric Neurology, Prague, Czech Republic
14. Medical School, Campus Bielefeld-Bethel, Bielefeld University, Germany
15. Department of Neuropathology, University Hospital Erlangen, Germany
16. King's College Hospital NHS Foundation Trust, Clinical Neuropathology, London, UK
17. Department of Child Neurology, UMCU Brain Center, Utrecht, the Netherlands
18. Department of Genetics, Center for Molecular Medicine, Utrecht, the Netherlands
19. Meyer Children's Hospital, Pathology Unit, Florence, Italy
20. Hospices Civils de Lyon, Dept. of Functional Neurology & Epileptology, Lyon, France
21. Lyon Neuroscience Research Center (INSERM U1028/CNRS UMR5292), Lyon, France
22. UCL NIHR BRC Great Ormond Street Institute of Child Health, London, UK
23. Hospital Sainte-Anne, Department of Neurology, Paris, France
24. Department of Pediatric Neurosurgery, Rothschild Foundation Hospital, Paris, France
25. UCL Queen Square Institute of Neurology & National Hospital for Neurology and Neurosurgery, London, UK
26. Developmental Biology and Cancer Programme, UCL GOS ICH, London, UK
27. Great Ormond Street Hospital for Children & Young Epilepsy, London/Lingfield, UK
28. Kuopio Epilepsy Center, Kuopio University Hospital, Kuopio, Finland
29. Department of Pathology, Kuopio University Hospital, Finland
30. IRCCS NEUROMED, Pozzilli, Italy
31. Department of Neurosurgery, Sapienza University of Rome, Rome, Italy
32. Foundation Rothschild Hospital, Pediatric Neurosurgery Department, Paris, France
33. Department of Paediatric Neurology, Ostrava Faculty Hospital, Ostrava, Czech Republic
34. Department of Epileptology, University of Bonn Medical Center, Bonn, Germany
35. Division of Pediatric Neurology, Severance Children's Hospital, Seoul, Republic of Korea
36. Graduate School of Medical Science and Engineering, KAIST, Daejeon, Republic of Korea
37. Ludwig-Maximilians University, Epilepsy Center, Munich, Germany
38. Department of Neurosurgery, Philipps University Marburg, Epilepsy Center Hessen, Germany
39. Motol Epilepsy Center, Charles University & Motol University Hospital, Prague, Czech Republic
40. Schoen Clinic, Center for Pediatric Neurology and Epileptology, Vogtareuth, Germany
41. PMU Salzburg, Research Institute "Rehabilitation, Transition, Palliation", Salzburg, Austria
42. University Hospital Freiburg, Epilepsy Center, Freiburg, Germany
43. University Hospital Freiburg, Department of Neurosurgery, Freiburg, Germany
44. Istanbul University – Cerrahpasa, Dept. of Neurology, Istanbul, Turkey
45. Swiss Epilepsy Center & University Hospital Zurich, Dept. of Neurology, Zurich, Switzerland
46. Meyer Children's Hospital, Neurosurgery Department, Florence, Italy
47. University of Florence, Florence, Italy
48. Goethe University Frankfurt, Epilepsy Center Frankfurt Rhine-Main, Germany
49. Philipps University Marburg, Department of Neurology, Epilepsy Center Hessen, Germany
50. Philipps University Marburg, Department of Neurosurgery, Epilepsy Center Hessen, Germany
51. University Hospital Leuven, Department of Neurology, Leuven, Belgium
52. University Hospital Leuven, Department of Neurosurgery, Leuven, Belgium
53. Fondazione IRCCS Carlo Besta, Epilepsy Unit, Milan, Italy
54. Medical University of Vienna, Center for Rare and Complex Epilepsies, Austria

55. Medical University of Vienna, Department of Neurosurgery, Austria
56. Hospital Ruber Internacional, Epilepsy Program, Madrid, Spain
57. Clinical Centre of Serbia, Laboratory for Neuropathology, Belgrade, Serbia
58. Clinical Centre of Serbia, Department for Epilepsy, Neurology Clinic, Belgrade, Serbia
59. University of Belgrade, Medical Faculty, Belgrade, Serbia
60. Maastricht University Medical Center, Department of Neurosurgery, Maastricht, Netherlands
61. Hospital Sant Joan de Déu, Paediatric Epilepsy & Neurosurgery Department, Barcelona, Spain
62. Genomic Medicine Institute, Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio, USA
63. Clinical and Experimental Neurology, Bambino Gesu' Children's Hospital, IRCCS, Rome, Italy

Nederlandstalige samenvatting

Epilepsiechirurgie is momenteel de enige potentieel curatieve behandeling voor patiënten met medicatieresistente focale epilepsie. De preoperatieve besluitvorming is traditioneel gebaseerd op een combinatie van de klinische aanvalsbeschrijving (semiologie), EEG en beeldvorming. Een aangetoonde structurele afwijking, bij voorkeur zichtbaar op MRI en passend bij de elektro-klinische gegevens, geldt daarbij als voornaamste uitgangspunt bij de beoordeling van geschiktheid voor epilepsiechirurgie.

Tot voor kort speelde genetische diagnostiek nauwelijks een rol in het prechirurgische traject. Alleen bij kinderen met epileptische encefalopathieën of een syndromaal beeld werd soms genetisch onderzoek overwogen, maar bij focale epilepsie zonder duidelijke syndromale kenmerken werd het doorgaans niet relevant geacht.

Dankzij nieuwe DNA-analysetechnieken, zoals next-generation sequencing, en een beter begrip van zowel aangeboren als verworven oorzaken van epilepsie is deze denkwijze veranderd. Bij kinderen met focale epilepsie worden inmiddels regelmatig genetische afwijkingen gevonden, ook wanneer MRI of weefselonderzoek geen afwijkingen laten zien. Daarmee verliest het traditionele onderscheid tussen een 'structurele' en een 'genetische' oorzaak klinische waarde, en krijgt genetisch onderzoek een steeds belangrijkere rol bij het begrijpen en classificeren van epilepsie.

De klinische meerwaarde van genetisch onderzoek binnen de prechirurgische besluitvorming is echter nog niet eenduidig vastgesteld. Belangrijke vragen zijn onder meer: wanneer is genetisch onderzoek geïndiceerd, hoe moeten genetische bevindingen worden geïnterpreteerd in relatie tot andere diagnostische gegevens, en heeft een genetische diagnose voorspellende waarde voor de uitkomst van epilepsiechirurgie? Ook is de vraag of genetisch onderzoek kan bijdragen aan een sneller en zorgvuldiger prechirurgisch traject, en hoe dit zich verhoudt tot het streven naar vroege interventie (vroege interventie leidt zoals bekend tot betere uitkomsten).
Dit proefschrift onderzoekt of, hoe en wanneer genetische diagnostiek zinvol kan worden ingezet in het prechirurgische evaluatietraject van patiënten met medicatieresistente focale epilepsie, en heeft tot doel de timing, selectie en uitkomst van epilepsiechirurgie te optimaliseren.

Deel I. De rol van genetisch onderzoek in epilepsiechirurgie

Hoofdstuk 2 schetst de genetische basis van epilepsie en de technologische ontwikkelingen die dit veld de afgelopen decennia hebben gevormd. Varianten in genen die betrokken zijn bij uiteenlopende biologische mechanismen (zoals ionkanaal- en synapsfunctie, transcriptieregulatie, metabolisme en het mTOR-signaalpad) kunnen ten grondslag liggen aan zowel focale als gegeneraliseerde epilepsieën, met of zonder structurele afwijkingen. De vroegere indeling in 'idiopathische' en 'symptomatische' epilepsieën is daarmee vervangen door een op etiologie gebaseerde classificatie. Hoewel inmiddels zeker is dat genetisch onderzoek waardevolle etiologische en prognostische informatie kan opleveren, maakt genetische diagnostiek nog nauwelijks deel uit van de prechirurgische evaluatie.

Hoofdstuk 3 beschrijft een systematische review van 36 studies naar chirurgische uitkomsten bij patiënten met medicatieresistente epilepsie en een genetisch bevestigde diagnose. De resultaten verschilden sterk per gen: chirurgie was vrijwel nooit succesvol bij varianten in ionkanaal- en

synapsgenen (zoals *SCN1A*); bij patiënten met varianten in het mTOR-signaalpad (zoals *DEPDC5*, *NPRL2* en *NPRL3*) blijkt epilepsiechirurgie daarentegen regelmatig succesvol. Deze bevindingen benadrukken de toegevoegde waarde van genetisch onderzoek tijdens de preoperatieve evaluatie.

Hoofdstuk 4 omvat een retrospectieve cohortstudie naar genetische diagnostiek bij 2385 patienten die tussen 1990 en 2016 in het UMC Utrecht voor epilepsiechirurgie werden geëvalueerd. Genetisch onderzoek werd vooral uitgevoerd bij MRI-negatieve patiënten, maar bij bijna de helft pas nadat reeds een chirurgisch besluit was genomen. Pathogene varianten werden met name gevonden in ionkanaal- en synapsgenen en in het mTOR-signaalpad. Aanvalsvrijheid na de operatie trad uitsluitend op bij patiënten met een mTOR-variant, zowel bij MRI-positieve als bij MRI-negatieve gevallen. Opvallend was dat bij sommige patiënten, waaronder kinderen met een *SCN1A*-variant (geassocieerd met een diffuus pathofysiologisch mechanisme), de genetische diagnose pas werd gesteld na invasieve diagnostiek. De bevindingen maken duidelijk dat genetische diagnostiek, mits tijdig uitgevoerd, van waarde kan zijn om patiënten met een grote kans op een gunstig resultaat te onderscheiden van patiënten bij wie invasieve procedures naar verwachting weinig opleveren.

Deel II. Genetische mechanismen van focale epilepsie

Hoofdstuk 5 beschrijft vier kinderen met familiaire, therapieresistente focale epilepsie en histologisch bevestigde focale corticale dysplasie (FCD); allen hadden een erfelijke variant in een GATOR1-gen (*DEPDC5*, *NPRL2* of *NPRL3*). Bij hen was het ziektebeloop (met ontwikkelingsachterstand, vroegere aanvang van de aanvallen en ongunstige postoperatieve uitkomst), ondanks typische kenmerken van FCD, ernstiger dan bij de ouder van wie de variant afkomstig was. Literatuuronderzoek bevestigde dit patroon van toenemende ernst in opeenvolgende generaties binnen families met GATOR1-gerelateerde epilepsie. Mogelijke verklaringen zijn incomplete penetrantie, second-hit varianten of mozaïcisme. Deze bevindingen suggereren dat familiair voorkomende GATOR1-gerelateerde FCD een genetisch gedefinieerde subgroep vormt met verhoogde klinische complexiteit. De uitkomst van epilepsiechirurgie is bij deze groep minder goed voorspelbaar, waardoor een zorgvuldige preoperatieve afweging extra belangrijk is.

In **hoofdstuk 6** onderzoeken we kiembaan- en somatische varianten in hersenweefsel van kinderen die een epilepsieoperatie ondergingen wegens (vermoedelijke) corticale aanlegstoornissen (*malformations of cortical development,* MCD). Bij FCD type II en hemimegalencefalie (HME) werden vooral varianten in het mTOR-signaalpad gevonden. Bij FCD type I, *mild malformation of cortical development* (mMCD) en *mild malformation of cortical development with oligodendroglial hyperplasia and epilepsy* (MOGHE) was de genetische achtergrond heterogener, met onder andere *SLC35A2*-varianten. Ook in weefsel zonder duidelijke histologische afwijkingen werden somatische varianten aangetoond, onder meer in *SLC35A2* en *DEPDC5*, vaak met een lage allelfrequentie (<5%). De resultaten benadrukken opnieuw het belang van gevoelige analysetechnieken, aangezien deze bijdragen aan een hogere diagnostische opbrengst en een betere genetische classificatie van zowel structurele als niet-structurele focale epilepsie.

Hoofdstuk 7 beschrijft een Europese multicentrische studie naar operatieve uitkomsten bij niet-lesionale patiënten (met normale MRI en normaal weefselonderzoek) met focale epilepsie. Bijna de helft van de patiënten met temporaalkwabepilepsie werd aanvalsvrij, waarbij degenen die ook een hippocampectomie hadden ondergaan de beste uitkomsten hadden. Extratemporale

chirurgie had daarentegen weinig succes. Ook invasieve monitoring hing, ongeacht de resectielocatie, samen met een slechtere uitkomst. Een langere epilepsieduur en aspecifieke gliose bleken eveneens ongunstige prognostische factoren. Genetisch onderzoek was slechts bij een klein deel van de patiënten verricht en had geen causale varianten opgeleverd. Deze resultaten laten zien dat resectieve epilepsiechirurgie ook bij afwezigheid van structurele afwijkingen zinvol kan zijn. Daarnaast laten de resultaten zien dat het tot op heden beperkte gebruik van genetische diagnostiek een beperking vormt, juist voor deze patiëntengroep. In de toekomst zullen deze patiënten baat hebben bij moleculair-genetisch onderzoek dat is gericht op betere etiologische classificatie en betrouwbare prognostische markers voor epilepsiechirurgie.

Deel III. Timing, uitkomst en klinische integratie van genetisch onderzoek

In **hoofdstuk 8** evalueren we factoren die de timing van epilepsiechirurgie beïnvloeden bij kinderen met MCD of laaggradige epilepsie-geassocieerde tumoren (LEAT). Kinderen met LEAT werden gemiddeld sneller geopereerd dan patiënten met MCD. Vroege medicatieresistentie, zichtbaarheid van een focus op de eerste MRI en een latere leeftijd bij aanvang van de epilepsieaanvallen hingen samen met een korter interval tot operatie. Een korter interval hing bovendien samen met een betere uitkomst. Bij een aanzienlijk deel van de patiënten die aanvankelijk als MRI-negatief werden beschouwd, werd in het expertisecentrum alsnog een focus geïdentificeerd. Deze bevinding benadrukt het belang van vroege verwijzing en beoordeling in gespecialiseerde centra.

Op basis van de in dit proefschrift beschreven onderzoeken kom ik tot de volgende conclusies:

1. Epilepsiechirurgie kan succesvol zijn bij patiënten met een genetische oorzaak, maar de uitkomst varieert afhankelijk van het onderliggende pathofysiologische mechanisme (hoofdstuk 3).
2. Genetisch onderzoek kan de klinische besluitvorming ondersteunen en dient derhalve consequent en in een vroeg stadium te worden overwogen bij alle patiënten die mogelijk in aanmerking komen voor epilepsiechirurgie (hoofdstuk 3 en 4).
3. Patiënten met GATOR1-gerelateerde familiaire FCD vormen een uitdagende subgroep, met een fenotype dat varieert tussen generaties en met gemiddeld minder gunstige chirurgische uitkomsten (hoofdstuk 5).
4. Bij sommige niet-lesionale patiënten werden somatische varianten met een lage allelfrequentie in hersenweefsel en mozaïcisme in bloed gevonden (hoofdstuk 6).
5. De prevalentie van somatische varianten bij patiënten met mMCD en bij patiënten met niet-lesionale epilepsie wordt vermoedelijk onderschat; deze bevinding benadrukt het belang van gevoelige sequencing-methoden met als doel een hogere diagnostische opbrengst en betere prechirurgische besluitvorming (hoofdstuk 6).
6. Zelfs zogenoemde niet-lesionele patiënten, bij wie op MRI en in weefselonderzoek geen causale afwijkingen konden worden vastgesteld en bij wie de onderliggende genetische oorzaken onvolledig zijn opgehelderd, kunnen langdurige postoperatieve aanvalsvrijheid bereiken, met name na mesiotemporale resecties (hoofdstuk 7).
7. Het geringe succespercentage bij extratemporale, niet-lesionale chirurgie benadrukt de noodzaak van nadere moleculaire karakterisering teneinde betrouwbare voorspellers van de uitkomst te identificeren (hoofdstuk 7).
8. Een kortere tijd tussen de diagnose van epilepsie en operatie is onafhankelijk geassocieerd met een grotere kans op aanvalsvrijheid na epilepsiechirurgie (hoofdstuk 8).

9. Bij afwezigheid van duidelijke MRI-afwijkingen verdient vroege verwijzing naar een expertisecentrum aanbeveling (hoofdstuk 8).

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Maurits

Curriculum vitae



Maurits Sanders werd op 18 augustus 1990 geboren te Breda. In 2004 behaalde hij het C-bijzonder examen piano aan De Nieuwe Veste. In 2007 verkreeg hij zijn gymnasiumdiploma aan het Mencia de Mendoza Lyceum. Een jaar later begon hij met de studie Geneeskunde aan de Universiteit Utrecht. Tijdens zijn studie deed hij onderzoek naar trigeminusneuralgie bij multipele sclerose. Daarnaast verrichtte hij wetenschapsstages in het gezondheidsrecht onder begeleiding van mr. Monique C.H.I. Biesaart en binnen de neuro-oncologie onder begeleiding van dr. Tom J. Snijders, gericht op focale status epilepticus bij gliomen. Eind 2015 behaalde hij het artsdiploma en startte aansluitend met de opleiding tot neuroloog in het UMC Utrecht. In 2017 begon hij onder begeleiding van prof. dr. Kees P.J. Braun, dr. Floor E. Jansen en dr. Bobby P.C. Koeleman aan zijn promotietraject, waarvan de resultaten in dit proefschrift worden beschreven. Naast zijn medische opleiding voltooide hij in 2022 een beroepsopleiding tot geluidstechnicus en muziekproducent, waarmee hij zijn passie voor muziek verder vormgaf. In 2024 startte hij na het afronden van zijn opleiding tot neuroloog een fellowship neuromusculaire ziekten, eveneens in het UMC Utrecht.

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