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Epilepsy in patients carrying COL4A1/COL4A2 mutations with focus on neuroimaging, epilepsy surgery and histology

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Summary

The *COL4A1* and *COL4A2* genes code for the α1 and α2 chain in Collagen IV, an essential component of the basement membrane (BM). The BM can be found ubiquitously in the human body. *COL4A1/-2* mutations lead to central nervous system (CNS) disorders, cataract and cystic renal disease. Disruption of the structural integrity of the BM in cerebral vessels causing intracerebral infarctions and bleedings is assumed to explain neurologic disorders. However, current understanding of epileptogenesis in patients carrying *COL4A1/-2* mutations is limited.
In this study, clinical course, neuroimaging, epilepsy surgery and its outcome, as well as histological findings in pediatric *COL4A1/-2* associated epilepsy were analysed. Moreover, mechanisms of epileptogenesis in patients with *COL4A1/-2* mutations were discussed. Particularly, we aimed to verify the hypothesis that cortical malformations may underly epileptogenesis in patients carrying *COL4A1/-2* mutations.

Patients with *COL4A1/-2* mutations and preoperative monitoring performed before the 28th February 2019 in Epilepsy Center for Children and Adolescents Vogtareuth were included. In total, nine patients were identified. Three out of them underwent epilepsy surgery. Last follow-up examination was performed 4 months to 10 years after preoperative monitoring. We analysed the clinical course and neuroimaging results in all our patients, as well as the histological findings in the three patients who underwent surgery, in order to better understand the complex disease pattern related to *COL4A1/-2* mutations.

In all patients of our cohort, who showed severe drug-resistant epilepsy with onset in the first year of life, a severe psychomotor developmental delay and motor dysfunction were observed. Primary microcephaly was found in seven out of nine cases, whereas in two cases, secondary microcephaly was found. Cataract was found in 4/9 patients and seems to be no obligate feature in patients with *COL4A1/-2* mutations.

Genetic analysis revealed in four cases *de novo* mutations (three *COL4A1* mutations, one *COL4A2* mutation), while in two cases (*COL4A1* mutation) maternal inheritance was shown. In three other cases, no genetic analysis of the parents was available. In the two cases of maternal inheritance, positive clinical history was found: in both cases gestational interruption in a previous gestation was reported, in one case stroke was reported. In our cohort, we found no hints for phenotypic changes depending on the position of the mutated amino acid.

Neuroimaging showed periventricular leukomalacia (PVL) and ventriculomegaly suggestive of intrauterine cerebral bleedings that occurred at different gestational stages in all patients. Remarkably, cortical malformations of various degree were found in all patients of our cohort.

After standardised presurgical evaluation three patients (patients 1-3) underwent epilepsy surgery. It should be pointed out that this is the first report on epilepsy surgery in patients with *COL4A2* mutations (patients 2 and 3). Two patients (patient 1 and 3) underwent a sagittal hemispherotomy, one patient (patient 2) a temporal resection with parieto-occipital disconnection. Surgical outcome after 12, 24 and 6 months respectively was classified as Engel IIIa in patients 1 and 2 and as Engel Ia in patient 3. Despite persisting seizures, neurocognitive improvement was observed in patients 1 and 2.

Histological findings in patient 1, who carries a *COL4A1* mutation, showed a focal cortical dysplasia (FCD) IIID and hippocampus sclerosis. In patients 2 and 3, carrying *COL4A2* mutations, a mild malformation of cortical development (mMCD) and gliotic changes were found. However, resected specimens available for analysis in patients 2 and 3 were limited due to operative approach (limited

temporal resection in temporo-occipital disconnection in patient 2, limited frontal resection in hemispherotomy in patient 3) and might not have been sufficient to provide conclusive diagnostic evidence.

We performed a literature review to reinforce the hypothesis that epileptogenesis in patients with *COL4A1/-2* mutations is related to malformations of cortical development (MCD). The results are summarised in tabular form with focus on clinical and neuroimaging findings. In 26 out of 32 patients with MCDs and *COL4A1/-2* mutations epilepsy or seizures were reported. The literature review underlines the relevance of MCDs for epileptogenesis in patients with *COL4A1/-2* mutations.
In a further review of literature we analysed prenatal injuries documented at different gestational age in 17 patients carrying *COL4A1* mutations and pointed out the relevance of the timing of the prenatal lesion for the further clinical course and development of epilepsy.

In literature, PVL, ventriculomegaly and microbleeds are the most often reported neuroimaging findings in patients with *COL4A1-/2* mutations. In our patients, these three findings were not always present at the same time. Importantly, cortical malformations were demonstrated in all patients of our cohort by neuroimaging and were confirmed by histologic findings (FCD III, mMCD) in patients 1-3. Cortical malformations might explain the unfavourable clinical course, the severe developmental delay and the limited improvement after epilepsy surgery in our patients.

Based on findings from our cohort and from performed literature review, we propose that two main mechanisms lead to the impairment of CNS development and neurological function: firstly, CNS impairment is the result of bleedings, vascular insults, and disseminated microbleeds, which are caused by vessel fragility due to vascular BM impairment in *COL4A1/-2* mutations. Secondly, malformations of cortical development, which can also result from impaired function of the pial BM during neuronal migration and cortical organisation in the fetal period, can contribute to the neurologic phenotype and the development of epilepsy in patients with *COL4A1/-2* mutations.

Depending on timing and localisation of injury, the different patterns of brain lesion in *COL4A1/-2* associated epilepsy can be explained. If the CNS injury pattern and the thereby caused epilepsy show a clear electro-clinico-anatomical correlation between an epileptogenic lesion and the ictal onset zone, a surgical approach can be considered. Based on our findings, epilepsy surgery in patients with *COL4A1/-2* mutations aims for seizure reduction, better chances for neurocognitive development and improved quality of life.

In future, studies with larger cohorts are needed for deeper understanding of pathogenetic mechanisms and further evaluation of therapeutic options in *COL4A1/-2* associated epilepsy.

Zusammenfassung

Die Gene *COL4A1* und *COL4A2* kodieren für die alpha-1 bzw. alpha-2 Untereinheit des Collagen Typ 4, welches ein essenzieller Bestandteil der Basalmembran darstellt, die ubiquitär im menschlichen Körper vorkommt. *COL4A1/-2* Mutationen führen zu Störungen im Zentralnervensystem (ZNS), Katarakt und zystischen Nierenerkrankungen. Bisher wurde eine vermehrte Gefäßbrüchigkeit bei Veränderungen der kapillären Basalmembran der cerebralen Gefäße und die dadurch verursachten Blutungsereignisse als ursächlich für die neurologischen Störungen betrachtet, die Mechanismen der Epilepsieentstehung bei dieser Erkrankung blieben jedoch weitgehend ungeklärt.
In dieser Arbeit wurden der klinische Verlauf, die neuroradiologischen Befunde, das epilepsiechirurgische Vorgehen mit dessen Outcome und die histologischen Befunde bei pädiatrischen Epilepsiepatienten mit *COL4A1/-2* Mutationen analysiert. Des Weiteren wurden Mechanismen der Epileptogenese diskutiert und insbesondere wurde die Hypothese überprüft, ob kortikale Aufbaustörungen bei Patienten mit *COL4A1/-2* Mutationen eine Rolle bei der Epilepsieentstehung spielen können.

Eingeschlossen wurden 9 Patienten mit nachgewiesener Mutation im *COL4A1* oder *-2* Gen, die im Epilepsiezentrum Vogtareuth ein prächirurgisches Monitoring vor dem 28. Februar 2019 erhielten. Von den neun eingeschlossenen Patienten wurden drei operiert. Die letzte Nachuntersuchung fand vier Monate bis zehn Jahre nach dem prächirurgischen Monitoring statt. Die Charakterisierung der Patienten nach klinischen und radiologischen sowie bei den drei operierten Patienten histologischen Kriterien erfolgte mit dem Ziel eines besseren Verständnisses dieses komplexen Krankheitsbildes.

Allen Patienten gemeinsam war ein früher Beginn der therapierefraktären Epilepsie, die spätestens im elften Lebensmonat begann. Alle Kinder wiesen außerdem eine damit einhergehende schwere psychomotorische Entwicklungsstörung und motorische Dysfunktionen auf. Bei 7/9 Patienten war eine primäre, bei 2/9 eine sekundäre Mikrocephalie auffällig. Nur in 4/9 Fällen wurde eine Katarakt detektiert, die somit kein obligater Bestandteil der klinischen Präsentation von *COL4A1/-2* Mutationen darzustellen scheint.

Die genetische Analyse ergab in vier Fällen *de novo* Mutationen (3 x *COL4A1*, 1 x *COL4A2*), in zwei Fällen eine maternale Vererbung (*COL4A1*). In den weiteren drei Fällen war keine elterliche Untersuchung durchgeführt worden. In den zwei Fällen maternaler Vererbung lagen bei der Mutter des Patienten in einem Fall ein Schlaganfall und in beiden Fällen ein Abort in der vorangehenden Schwangerschaft vor. Es fanden sich in dieser Kohorte keine Hinweise für phänotypische Unterschiede in Abhängigkeit von der Aminosäurenposition der Mutation.

In der Bildgebung zeigten alle Patienten eine periventrikuläre Leukomalazie und eine Ventrikulomegalie als Zeichen einer mehrzeitigen intrauterinen Hirnblutung. Radiologische Hinweise auf das Vorliegen einer kortikalen Malformation wurden in allen Fällen in unterschiedlichem Ausmaß gefunden.

In drei von neun Fällen erfolgte nach standardisierter prächirurgischer Diagnostik ein epilepsiechirurgisches Vorgehen, in zwei von drei Fällen erstmalig bei Patienten mit *COL4A2* Mutationen. Zwei Patienten erhielten eine vertikale, parasagittale Hemisphärotomie (Patient 1 und 3), ein Patient (Patient 2) eine temporale Resektion mit parieto-occipitaler Diskonnektion. Das Outcome wurde nach der Engel-Klassifikation bei Patient 1 und 2 nach 12, 24 Monaten mit IIIa und bei Patient 3 nach 6 Monaten mit Ia bewertet. Trotz anhaltenden Anfällen konnten bei Patient 1 und 2 deutliche Entwicklungsfortschritte beobachtet werden.

Die histologischen Analysen zeigten bei Patient 1 (mit *COL4A1* Mutation) eine fokale kortikale Dysplasie (FCD) IIID sowie eine Hippocampussklerose, bei den Patienten 2 und 3 (mit *COL4A2*

Mutation) eine milde Malformation der kortikalen Entwicklung (mMCD) sowie gliotische Veränderungen. Es muss allerdings berücksichtigt werden, dass aufgrund der Art des operativen Vorgehens (kleine temporale Resektion bei temporo-occipitaler Diskonnektion bei Patient 2 und kleine frontale Resektion bei Hemisphärotomie bei Patient 3) in den zwei *COL4A2* Fällen nur wenig und ggfs. nicht ausreichend repräsentatives Material untersucht werden konnte.

Um die Hypothese zu untermauern, dass die Epileptogenese bei Patienten mit *COL4A1/-2* Mutationen in Zusammenhang mit kortikalen Aufbaustörungen steht, wurde eine ausführliche Literaturrecherche durchgeführt und daraus die radiologischen und klinischen Befunde tabellarisch zusammengefasst. Es wurden 32 Fälle mit kortikalen Dysplasien und *COL4A1/-2* Mutationen identifiziert, bei denen in 26 Fällen Epilepsie oder Anfälle beschrieben wurden. Diese Literaturrecherche unterstützte somit die mögliche Relevanz von Malformationen der kortikalen Entwicklung für die Epileptogenese bei Patienten mit *COL4A1/-2* Mutationen.
In einer weiterführenden Literaturrecherche wurden 17 Patienten mit *COL4A1* Mutationen und zu verschiedenen Gestationszeitpunkten dokumentierten vorgeburtlichen Hirnläsionen analysiert, wodurch die Relevanz des Zeitpunktes der pränatalen Schädigung für den weiteren klinischen Verlauf aufgezeigt werden konnte.

In dieser Arbeit konnte gezeigt werden, dass die bei *COL4A1/-2* Mutationen bisher am häufigsten beschriebenen MRT-Auffälligkeiten (periventrikuläre Leukomalazie, Ventrikulomegalie und Mikroblutungen) in dieser Kombination nur bei einem Teil der Patienten vorkommen. Bei allen Patienten in dieser Kohorte zeigten sich jedoch radiologische Hinweise auf kortikale Aufbaustörungen, die durch die histologischen Ergebnisse (FCD IIID, mMCD) bei den Patienten 1-3 untermauert werden konnten. Diese könnten den ungünstigen Krankheitsverlauf und die schwere der Entwicklungsstörung der Patienten erklären, sowie das nicht anfallsfreie Outcome bei zwei Patienten nach epilepsiechirurgischem Eingriff.

Auf der Basis der durchgeführten Literaturrecherche und der Befunde in dieser Kohorte wird vorgeschlagen, dass die ZNS-Entwicklungs- und Funktionsstörung bei *COL4A1/-2* Mutationen zum einen durch Blutungen, vaskuläre Insulte und durch disseminierte Mikroblutungen bedingt wird, die bei veränderter Gefäßarchitektur infolge der Basalmembranveränderung entstehen. Zum anderen können auch Malformationen der kortikalen Entwicklung, die auch durch eine gestörte Funktion der pialen Basalmembran während der neuronalen Migration und kortikalen Organisation in der Fetalzeit bedingt sind, zum Phänotyp der Erkrankung und zur Entstehung der Epilepsie beitragen.

In Abhängigkeit von dem Zeitpunkt und der Lokalisation der ZNS-Läsionen können verschiedene Schädigungsmuster bei *COL4A1/-2* assoziierter Epilepsie erklärt werden. Zeigt sich bei hoch selektierten Patienten eine klare elektro-kliniko-anatomische Korrelation zwischen einer epileptogenen Läsion und der Anfallsursprungszone, kann ein epilepsiechirurgischer Ansatz in Erwägung gezogen werden. Basierend auf unseren Ergebnissen, zielt der chirurgische Ansatz bei Epilepsiepatienten mit *COL4A1/-2* Mutationen auf Anfallsreduktion, bessere Voraussetzung für kognitive Entwicklung und zu erreichende Lebensqualität ab.

Weitere Studien über größere Kohorten sind nötig, um die Pathogenese und die therapeutischen Optionen bei den schweren Manifestationen dieser Erkrankung weiter aufzuschlüsseln.

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1 Introduction

COL4A1 and *COL4A2* mutations can lead to intracerebral hemorrage, epilepsy and a variety of neurological deficits as well as ocular and renal impairment (Gould et al. 2005; Favor et al. 2007; Sibon et al. 2007; Plaisier et al. 2007; Meuwissen et al. 2015; Zagaglia et al. 2018). These phenotypes are probably caused by abnormal structure and function of collagen IV, the main component of the basement membrane (Pöschl et al. 2004; Gould et al. 2005; Plaisier et al. 2007; Jeanne and Gould 2017).
In our study, clinical findings and epilepsy surgery in children with *COL4A1* or *-2* mutation related epilepsy are evaluated. Epilepsy semiology, family history, as well as findings in magnetic resonance imaging (MRI) and electroencephalography (EEG) are analysed. Histology findings from resected specimens give insights in cortical and white matter changes in patients harboring *COL4A1/-2* mutations and help to discuss mechanisms of central nervous system (CNS) damage.

At the beginning of this chapter (sections 1.1 - 1.2), the aim of the study and current knowledge on *COL4A1/-2* mutations are presented. In the sections 1.3 - 1.5, fundamental aspects on epilepsy, epilepsy surgery and related histological findings are introduced.

1.1 Aim of the study

The aim of the study is to analyse clinical history and epilepsy semiology in relation to neuroimaging findings and histology to better understand *COL4A1/-2* related disease.

Further goals of the study consist in identifying features of patients who can be candidates for epilepsy surgery and in evaluating the clinical outcome after epilepsy surgery in drug-resistant *COL4A1-/2* related childhood epilepsy.

Following questions are topic of investigation:

I. Which phenotype is observed in *COL4A1 /-2* epilepsy patients?

- I.a Clinical and family history of the patients: what are common findings?
- I.b What are typical neuroimaging findings?
- I.c Timing of brain injury: at which developmental stages are lesions observed?
- I.d What are diagnostic clues in *COL4A1/-2* epilepsy patients?

II. How can we define candidacy for epilepsy surgery in *COL4A1/-2* related epilepsy?

III. What is the outcome of epilepsy surgery in patients with *COL4A1/-2* mutations?

IV. What is the pathophysiology underlying *COL4A1/-2* related epilepsy?

- IV.a Histological findings: Which clues about pathophysiology can be found?
- IV.b What are possible mechanisms of epileptogenesis?

This study will help to define the clinical phenotype and therapeutic options in *COL4A1/-2* related epilepsy. Moreover, we aim to get further insights into the pathophysiology of *COL4A1-/2* related disease.

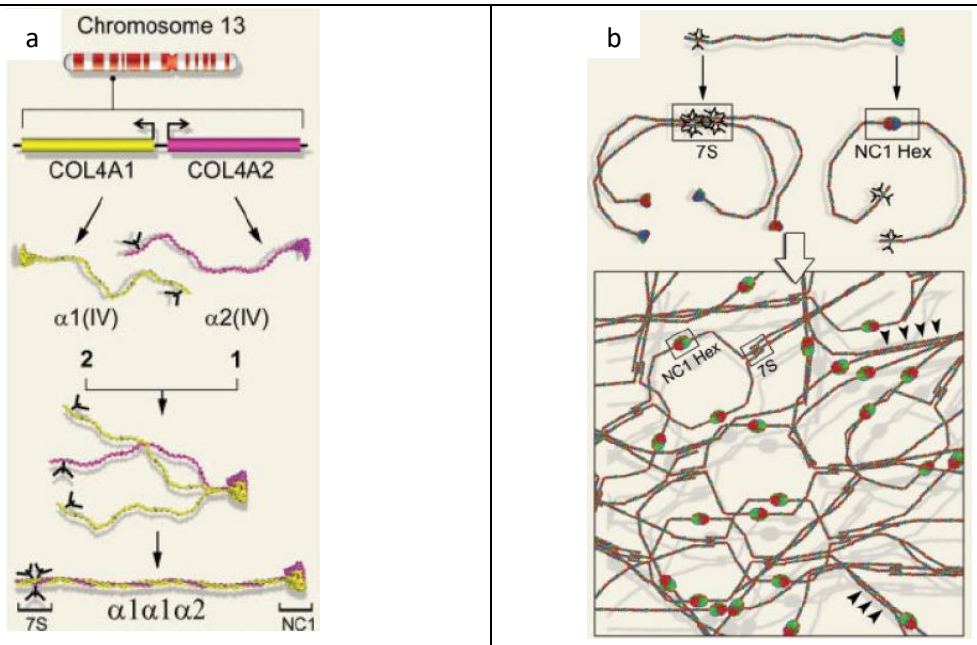
1.2 *COL4A1* and *-2* mutations

1.2.1 Collagen IV and *COL4A1/-2*

Collagen IV is an elastic, non-fibrillous protein and together with laminin, nidogen, and proteogylcans a main component of the basement membrane (BM). BMs are extracellular matrices underlying epithelial and endothelial cell layers and surrounding mesenchymal cells, with essential functions for tissue stability, compartmentation, cell-matrix interaction and cell migration (Vasudevan et al. 2010; Yurchenco 2011). Collagen IV molecules build complex macromolecular networks, consisting in various combinations of α1-α6 chains, which are coded by six distinct human genes.

The two genes *COL4A1* and *-2* on human chromosome 13 code for heterotrimeres with stochiometry α1α1α2. *COL4A1* and *-2* are expressed broadly in BMs, whereas *COL4A3-6* show a tissue-specific expression (Kefalides 1971; Hudson 1993; Leinonen 1994; Sado et al. 1998; Khoshnoodi et al. 2008). Collagen IV´s involvement in Alport Syndrome[1] and Goodpasture Syndrome[2] is well known, reviewed in (Khoshnoodi et al. 2008). For involvement of collagen in other diseases, see Attachments 9.2.

The collagen IV alpha chain consists of three domains: the aminoterminus, the non-collagenous carboxyterminus (NC1), and the triple helix (THX) domain. Alpha chains build heterotrimers (Fig.1a), which then assemble to tetramers in the extracellular matrix through crosslinking and self-folding. Irregular polygonal networks are formed (Fig.1b). The NC1 domain is considered the assembly director of the collagen molecule. The THX domain is constituted by highly conserved chains of Glycine and two variable amino acids (Gly-Xaa-Yaa) and is interrupted by non-collagenous domains, which give flexibility to the macromolecule (Khoshnoodi et al. 2008), see Figure *1*.



a. Schematic reconstruction. The paired *COL4A1*- and *2* genes are transcribed and translated into α1 and α2 chains. Chains show an aminoterminal 7S domain, a long-collagenous domain and a carboxyterminal non-collagenous domain (NC1). Heterotrimers are composed of two α1 and one α2 chains. They assembly beginning at the NC1 domain and then supercoiling the collagenous triple helical domains.

b. A heterotrimer can interact through its aminoterminus to form a tetramer (left arrow) or through its carboxyterminus to form a dimer (right arrow). Complex interactions lead to three-dimensional networks, which are enforced by supramolecular twisting and lateral associations of collagenous triple helical domains (arrow heads).

Figure 1. Collagen IV.
From (Khoshnoodi et al. 2008), with permission.

[1] Alport Syndrome: Hereditary nephritis, hematuria and sensorineural deafness, first described 1927 by Sir Alport; caused by mutations in *COL4A3/4/5* gene, reviewed in (Khoshnoodi et al. 2008).

[2] Goodpasture Syndrome: Antibodies against α3-chain of Collagen IV lead to rapid-progressive glomerulonephritis and lung hemorrhage, reviewed in (Khoshnoodi et al. 2008).

1.2.2 Phenotype in *COL4A1/-2* mutations

1.2.2.1 *COL4A1* mutations

A randomly generated *COL4A1* mutation in a mouse model leading to porencephaly[3] and intracerebral hemorrhage was described 2005. While homozygous mice died after mid-embryogenesis, about half of heterozygous mice survived postnatal day 1 (Gould et al. 2005). Before, a study group had created null allele mice for *COL4A1/2*, ablating both α1 and α2 chains to investigate basement membrane (BM) function. Homozygous mutation lead to embryonic death of the mice. This study had showed essential function of *COL4A1* and *-2* for structural integrity of the BM (Pöschl et al. 2004).

A wide phenotypic range has been documented in clinical reports since then.

Among neurological phenotypes, fatal intracerebral hemorhage while receiving anticoagulant therapy, fatal traumatic cerebral hemorrhage in adults (Gould et al. 2006), sudden-onset hemorrhagic stroke in one adolescent (Shah et al. 2010), recurrent childhood-onset stroke, infantile hemiplegia, focal epilepsy (Shah et al. 2012), schizencephaly, porencephaly (Yoneda et al. 2013; Smigiel et al. 2016), cerebral aneurysms, migraine (Lanfranconi and Markus 2010) and intrauterine stroke (Lichtenbelt et al. 2012; Garel et al. 2013; Durrani-Kolarik et al. 2017) were observed in patients. *COL4A1* mutation was identified as a monogenic cause of small-vessel-disease with mean-onset age of stroke of 36 years (Lanfranconi and Markus 2010). Also juvenile-onset dystonia and mental retardation in a patient with caudate nucleus hemorrhage have been reported (Hatano et al. 2017).

In a recent review severe developmental delay, epilepsy, intellectual disability, motor impairment of pyramidal and extrapyramidal systems were recognised as main neurologic features in this pathology (Zagaglia et al. 2018).

COL4A1 mutations can lead to a multisystem disorder. Axenfeld-Rieger-Anomaly[4], ophthalmological abnormalities (Sibon et al. 2007; Coupry et al. 2010) and HANAC Syndome[5] (Plaisier et al. 2007) were the first extra-CNS findings being associated with *COL4A1* mutation. Since then, kidney involvement has been shown to be a characteristic feature in *COL4A1* mutations. Recently, in familial polycystic kidney disease negative for *PKD2* mutation, *COL4A1* mutation was found (Cornec-Le Gall et al. 2018).

A research group demonstrated ocular dysgenesis, neuronal localisation defects, and congenital myopathy in *COL4A1* mice. They then identified *COL4A1* mutations in two patients with Muscle-Eye-Brain disease/Walter-Warburg-Syndrome[6] (Labelle-Dumais et al. 2011).

Ocular impairment comprising congenital cataract, anterior chamber dysgenesis and retinal vessel tortuosity was shown to be the most common extra-CNS phenotype (Zagaglia et al. 2018). Retinal hemorrhage has been recently suggested as a screening tool for intracerebral hemorrhage (Ratelade et al. 2018).

Also the involvement of the cardiac system (Yang et al. 2016), the hemopoietic system with hemolytic anemia (Yoneda et al. 2013) or hemolytic jaundice (Takenouchi et al. 2015; Tomotaki et al. 2016) and of the pulmonary system with alveolar hemorrhage in a neonate (Abe et al. 2017) have been described in association with *COL4A1* mutations. Moreover, relevance in gestation has been discussed (Zagaglia et al. 2018), since *COL4A1/2* are preeclampsia susceptibility genes (Yong et al.

[3] Porencephaly: can be described as “a neurologic disorder involving fluid-filled cavities in the brain” (Yoneda et al. 2012) often leading to hemiplegia, but also to epilepsy. Varying definitions and etiologies have been proposed for this condition.

[4] Axenfeld-Rieger anomaly: microcornea, congenital or juvenile cataract, increased intraocular pressure, iris hypoplasia, retinal detachment, optic nerve excavation.

[5] HANAC: Hereditary angiopathy with nephropathy, aneurysms and muscle cramps.

[6] “Muscle-eye-brain disease (MEB) and Walker-Warburg-Syndrome (WWS) are devastating childhood diseases that belong to a subgroup of congenital muscular dystrophies (CMDs) characterized by ocular dysgenesis, neuronal migration defects, and congenital myopathy“. Alteration in dystroglycan gylcosylation and *COL4A1* mutations are possible etiologies (Labelle-Dumais et al. 2011).

2014).
Multi-system involvement can be due to relevance of BM in different organs, even if pathogenesis and genotype-phenotype correlation ist still not profoundly understood.

1.2.2.2 *COL4A2* mutations

COL4A2 mutations have been first documented in mice with eye, brain, kidney and vascular defects (Favor et al. 2007). Reports on patients carrying *COL4A2* mutations with porencephaly (Yoneda et al. 2012; Verbeek et al. 2012; Ha et al. 2016), hemorrhagic stroke (Jeanne et al. 2012) and microcephaly (Verbeek et al. 2012) were published. A phenotypical range overlapping with *COL4A1* phenotype has been described since then (Meuwissen et al. 2015; Zagaglia et al. 2018). An identical *COL4A2* mutation, leading to hemiplegia and porencephaly, but also found in asymptomatic carriers in one family over four generations (Vilain et al. 2002) and as a *de novo* mutation leading to migraine and small aneuryms in a young man was reported (Kollmann et al. 2016). A report about a family showing neurologic disease over several generations including hemiplegia, epilepsy, porencephaly as well as a new disease entity including juvenile idiopathic arthritis has been published (McGovern et al. 2017). However, *COL4A2* mutations have been more rarely described than *COL4A1* mutations and thus phenotypical appearance is not as well known.

1.2.3 Prevalence

Prevalence of *COL4A1/-2* mutations in general population and in childhood related epilepsy is not known. A recent review could identify 123 patients with 69 different *COL4A1/-2* mutations previously reported in literature (Zagaglia et al. 2018). Among these patients, 55 showed epilepsy. Moreover, further 44 new patients with *COL4A1/-2* mutations, including 38 with epilepsy were described in this review (Zagaglia et al. 2018).
The following studies provide information on the prevalence of *COL4A1/-2* mutations in specific patient populations: in a study searching for *COL4A1* mutations in sporadic intracerebral hemorrage in adults, two putatively causative *COL4A1* mutations out of 96 screened patients were found (Weng et al. 2012). Among 41 preterm neonates with intraventricular hemorrhage, one maternal inherited *COL4A1* mutation (duplication) was found in dizygotic twins (Bilguvar et al. 2009).
In testing 183 patients with neuroimaging showing porencephaly or infantile hemorrhage, 24 *COL4A1/-2* mutations were found, and a prevalence of about 13% in similar patient populations was hypothetised (Meuwissen et al. 2015).
It has been speculated, that prevalence of *COL4A1/-2* mutations may be underestimated, particularly in patients with mild neurologic phenotype or unspecific MRI findings (Zagaglia et al. 2018) and in adult population with cerebrovascular disease (Meuwissen et al. 2015). The authors suggested, that increased awareness for neurologic phenotype related to *COL4A1/-2* mutations is needed (Meuwissen et al. 2015; Zagaglia et al. 2018).
Importantly, not all mutation carriers develop *COL4A1/-2* related disease (see also Ch. 1.2.5).

1.2.4 Epilepsy and epilepsy surgery in patients with *COL4A1/-2* mutations

A recent review showed that among 123 patients with *COL4A1/2* mutations published till then, in 55 epilepsy was mentioned, but in only 16 out of 55 the epilepsy phenotype had been described (Zagaglia et al. 2018). Epilepsy is therefore a quite common but not well understood manifestation in patients with *COL4A1/-2* mutations. Moreover, selection bias in literature needs to be considered, because often, only patients with severe MRI-changes and multisystem disease are screened for *COL4A1/2* mutation (Zagaglia et al. 2018).
The reported epilepsy phenotype includes infantile spasms, myoclonic jerks, focal seizures with secondary generalization (Shah et al. 2012), postictal hemiparesis after status epilepticus (Leung et al. 2012) and epileptic encephalopathy (Hino-Fukuyo et al. 2017) in patients with *COL4A1* mutations, as well as seizures with vomiting, nausea followed by motionless arrest (Yoneda et al. 2012),

refractory focal seizures and status epilepticus (Ha et al. 2016) in patients with *COL4A2* mutations. Mean age at onset of epilepsy was 15 months and the most common phenotype was found to be focal-onset seizures (in 73.7%) among 38 new presented patients with epilepsy and *COL4A1/-2* mutations (Zagaglia et al. 2018). Epileptic spasms and generalised tonic-clonic seizures were also observed. Among 66.6% of the patients in this cohort, epilepsy was drug-resistant. In EEG, focal epileptic discharges but also generalised slowing and sharp-waves were described in the cohort. Focal epileptiform discharges were associated to a MRI lesion in 46.4% of the patients. This review interestingly identified a subgroup (approximatively 10%) among the new reported patients and among further already published patients with epilepsy and *COL4A1/-2* mutations, wherein epilepsy was the main clinical feature and was accompanied by only diffuse MRI abnormalities (Zagaglia et al. 2018).

To date, three cases of epilepsy surgery in patients with *COL4A1* mutations have been reported. In the first case, a corpus callosotomy in a 6-year-old girl with structural focal epilepsy lead to a seizure-free period of 4 months (Papandreou et al. 2014). In the second case, after an initial corpus callosotomy with 1 year and 8 months age, a second surgical procedure consisting in a vertical parasagittal hemispherotomy was performed in the same patient at age 2 ½ years due to epileptic encephalopathy and hemihypsarrhytmia, and a seizure-free period of 1 year and 6 months was achieved (Hino-Fukuyo et al. 2017). In the third case, one patient received resection of a left temporo-occipital dysplasia at 21 months age and was seizure-free one year after surgery (Zagaglia et al. 2018). In the last case, histology was shortly described (see Table *9*). To our knowledge, no case with *COL4A2* mutation and epilepsy surgery was reported up to now.

1.2.5 Genetic aspects of *COL4A1/-2* mutations

In patients, only heterozygous mutations have been observed. Homozygous mutations have been shown to be lethal in mice with null allele for the *COL4A1/-2* locus (Pöschl et al. 2004) and in a *COL4A1* mutant mouse model generated by random mutagenesis (Gould et al. 2005). A recent extensive genotype-phenotype correlation study showed dominant inheritance in all so far described mutations (Jeanne and Gould 2017). According to this study, the number of inherited and *de novo* mutations seems to be approximately equal. This study also showed that glycine substitutions within the triple helical domain represent the most common class of mutations (Jeanne and Gould 2017). Mutations of glycines are supposed to cause disruption of triple-helix assembly and thus protein dysfunction (Khoshnoodi et al. 2008). Glycine has been observed to be mostly substituted by a charged amino acid. The location of the mutation and not the amino acid seem to influence severity of the disease. Location near the aminoterminus was likely to cause milder pathology, while location near the carboxyl end of the triple helix caused more severe cerebrovascular disease. Mutations affecting the integrin binding sites showed greater likelyhood of causing nephropathy and myopathy in patients, suggesting functional relevance of these binding sites (Jeanne and Gould 2017). Interestingly, penetrance of phenotypes is very variable, and could be influenced by location of the mutation in the gene, genetic modifiers[7] or environmental influences (Labelle-Dumais et al. 2011; Jeanne and Gould 2017).

Genetic mosaicism was also discussed as possible cause for varying penetrance. Varying penetrance[8] and expressivity[9] of the mutation were observed, and *COL4A1* mutations have been defined as

[7] Genetic modifier: Mutations in a single gene may be insufficient to explain different phenotypes. Loss or alteration of contiguous genes or of proteins (such as transporter or activator proteins and chaperones) are recognised as a source of phenotypic variation (Slavotinek 2003).

[8] Penetrance: "Percentage of individuals with a given genotype who exhibit the phenotype associated with that genotype" (Griffiths AJF, Miller JH, Suzuki DT, et al. 2000).

[9] Expressivity: "Expressivity measures the extent to which a given genotype is expressed at the phenotypic level. Different degrees of expression in different individuals may be due to variation in the allelic constitution of the rest of the genome or to environmental factors." (Griffiths AJF, Miller JH, Suzuki DT, et al. 2000).

"pleiotropic"[10] (Labelle-Dumais et al. 2011).
A recent study observed increasing penetrance in maternally inherited mutations. The authors proposed, maternal uterus vasculature impairment may lead to prenatal complications and thus increased disease severity in the fetus (Zagaglia et al. 2018).
It was suggested, that due to biological differences in the collagen chains (one heterotrimer consists of two α1 chains together with only one α2 chain), *COL4A2* mutations may lead to a milder phenotype than *COL4A1* mutations. However, since more patients with *COL4A1* than with *COL4A2* mutations have been described, an ascertainment bias has been suggested (Jeanne and Gould 2017).

1.2.6 Pathogenic mechanisms in *COL4A1/-2* mutations

1.2.6.1 CNS Involvement

In the brain, basement membranes (BM) are found around blood vessels and under the pial surface. They show a thickness of 20-200 nm and represent the main component of the extracellular matrix in the brain (Choi 1994; Halfter et al. 2002; Engelhardt and Sorokin 2009). Collagen IV along with BM was present only in three locations in mice brain: in blood vessels, beneath the pia mater and the choroid plexus. Here, α1 and 2 chains were found ubiquitously (Urabe et al. 2002).

1.2.6.1.1 Hemorrhage

BM instability with the consequence of vascular fragility and increased bleeding is the most obvious mechanism of damage to the CNS. The role of an intact BM for vascular stability had already been shown in a mouse model with deficient alleles for both *COL4A1* and *-2* (Pöschl et al. 2004). Other studies showed increased intracerebral hemorrhages (ICH) in *COL4A1* mice mutants correlating with environmental stress, such as birth trauma (Gould et al. 2005; Gould et al. 2006). Here, the authors proposed that pressure on the head during birth may lead to ICH and thus to porencephaly, since no severe ICH were found after surgical delivery of mouse mutants. Interestingly, in their study ICH was often found in basal ganglia. The authors observed a pattern similar to small-vessel-disease and hypothetised that BM defects weaken vessels branching at large angles, such as the lenticulostriatal arteries supplying the basal ganglia (Gould et al. 2006).
In a study by Ratelade et al. on *COL4A1* mutant mice published in 2018, it was suggested that microhemorrhages and macrohemorrhages arise due to different pathomechanisms. The authors reported decreased COL4A1 and -2 expression in cerebral vessel BM, as well as smooth muscle cell loss in deep arteries. Transient but generalised permeability increase of capillaries in relation to microhemorrhages was found in blood-brain-barrier of *COL4A1* mutant mice (Ratelade et al. 2018).

1.2.6.1.2 Corticogenesis and epileptogenesis

After recognizing several patients showing pathologies related to malformations of cortical development and neuronal displacement, such as gyral abnormalities, schizencephaly and porencephaly, impairment of corticogenesis in *COL4A1/2* mutations was suggested, though the precise mechanism remained unknown (Labelle-Dumais et al. 2011; Yoneda et al. 2013). One group was able to show abnormal neuronal clustering in one patient´s histology (Yoneda et al. 2013).
The important role of an intact pial BM for neuronal migration during brain development has been demonstrated in several studies: radial glia cells, which function as a scaffold for radial neuronal migration from the periventricular germinal zone to the cortex, are anchored on the pial BM, and gaps in the pial BM were shown in animal models to lead to heterotopias and abnormal neuronal migration (Choi 1994; Halfter et al. 2002; Haubst et al. 2006; Hu et al. 2007), see Attachments, 9.4.
The particular role of *COL4A1* and *-2* in cortical development, had been first investigated by Pöschl et al. in a mouse model with deficient allel for *COL4A1* and -2, in which disrupted pial surface and neuronal ectopias were found (Pöschl et al. 2004).

[10]Pleiotropic gene: Gene that influences multiple traits (Paaby und Rockman 2013).

COL4A1 mutations have been found to cause neuronal dyslocalisation in a mutant mouse model for Muscle-Eye-Brain Disease/Walker-Warburg Syndrome (Labelle-Dumais et al. 2011), which consists of a neuronal migration disorder during the late migration period (Barkovich et al. 2012).
Epileptogenesis in patients with *COL4A1/-2* mutations has not been sufficiently investigated yet. Epileptogenicity of hemosiderin and blood products has been demonstrated (Rosen A. D. and Frumin N. V. 1979; Ueda et al. 1998; Roelcke et al. 2013) and could explain epileptogenic focus in hemorrhagic lesions. John et al. speculated on physiological changes due to *COL4A1/-2* mutation (John et al. 2015). Our discussion of possible mechanisms involved in epileptogenesis in *COL4A1/-2* related epilepsy is presented in Ch. 4.4.2.

1.2.6.2 Extra-CNS Involvement

1.2.6.2.1 Kidney and ocular phenotype

Renal phenotype in *COL4A1* mutations has been proposed as resulting from defects in BM of Bowman´s capsule, renal capillaries and tubuli leading to hematuria and formation of renal cysts (Plaisier et al. 2007). Glomerular BM is mainly composed of collagen α3 α4 α5 after embryogenesis and thus not supposed to be affected by *COL4A1/2* mutations, even if cases of thinning of glomerular BM have been reported (Gale et al. 2016).
Congenital non-syndromal cataract was shown in patients with *COL4A1* mutations, and cellular or endoplasmic reticulum (ER) stress in the lens cells following improper collagen folding was supposed (Xia et al. 2014). Another group found vascular defects and increased vessel proliferation leading to retinopathy in *COL4A1* mutant mice (Alavi et al. 2016). Optic nerve hypoplasia is supposed to partly derive from mislocalization and pathologic apoptosis of radial glial cells during development (Labelle-Dumais et al. 2011).
Photoreceptor degeneration, associated with vascular abnormalities and reactive gliosis, has been observed and a role of impairment of metabolic support to neuronal cells has been discussed in a mouse model of HANAC Syndrome (Trouillet et al. 2017).

1.2.6.2.2 Extracellular matrix and cellular damage

Impairment of extracellular matrix protein-protein communication was suggested, particularly if the mutation is affecting a binding site of collagen IV to other extracellular proteins (Labelle-Dumais et al. 2011).
On the cellular level, ER stress caused by accumulation of unproperly folded collagen has been documented and could lead to cellular dysfunction (Gould et al. 2007; Jeanne et al. 2012). Decreased COL4A1 secretion was shown in mice with a *COL4A1* mutation related to intracranial hemorrhage (Gould et al. 2005) and in a human cellular model for *COL4A1* mutations occurring in the triple helix-forming domain (Weng et al. 2012). Intracellular stress due to accumulation of misfolded COL4A1 protein, or decreased COL4A1 secretion into the BM were supposed to be pathogenic factors in cerebrovascular disease related to *COL4A1/-2* mutations (Gould et al. 2005; Weng et al. 2012). Chaperones for protein folding were proposed as possible therapeutic approach (Weng et al. 2012). Chemical chaperone treatment performed in cells with *COL4A2* mutation reduced ER stress and intracellular COL4A2 accumulation (Murray et al. 2014). Oral chemical chaperone treatment with 4-sodium phenyl butyric acid (PBA) to reduce ER stress was performed in *COL4A1* mutant mice (Jones et al. 2018). Here, severity, but not number of intracerebral hemorrages was reduced. Interestingly, neuroglial inflammatory activation following hemorrhage was found to be reduced after treatment. Anyway, increased collagen deposition in BM did not increase BM stability and did not improve eye and kidney defects (Jones et al. 2018).

1.2.7 Neuroimaging findings and underlying fetal brain injury in patients with *COL4A1/-2* mutations

In patients with *COL4A1/-2* mutations, neuroimaging may differ strongly depending on features of underlying injury. The fetal brain reacts depending on cause, timing and location of the injury, so that neuroimaging findings can give hints on the past pathologic event.

1.2.7.1 Venous vs arterial infarction

On the one hand, a thrombotic or embolic event leads to a lesion in the area of a major cerebral artery branch. General hypotension and hypoperfusion lead to diffuse damage in the intervascular boundary zones. On the other hand, venous infarction can lead to hemorrhages in the anterior temporal lobe, or with frontal or parietal involvement.
COL4A1 mutations seem to cause hemorrhage looking "identical to hemorrhagic venous infarction" (Barkovich and Raybaud 2012, Ch.4). In addition, *COL4A1* mutation has been proposed as a major cause of perinatal arterial ischemic stroke (Volpe 2018, Ch. 21) and is an arteriopathy among the main causes for fetal brain injury (Barkovich and Raybaud 2012, Ch.4). Genetic counselling for *COL4A1* mutation was considered, if both hemorrhagic and ischemic fetal brain injuries were found (Khalid et al. 2018). In the first case of a fetal diagnosis of *COL4A1* mutation, supratentorial hemorrhage and cerebellar lesion were reported as leading to the diagnosis (Lichtenbelt et al. 2012). Thus, according to the above mentioned literature, features of venous infarction as well as features of arterial infarction may be present in patients with *COL4A1/-2* mutations.

1.2.7.2 Timing of injury

During the second trimester of gestation, necrotic tissue is reabsorbed and leads to formation of a cyst (which can also be defined as a *porencephalic cyst* or *cystic encephalomalacia*). In the third trimester, the fetal brain starts to develop the ability to react to injury with astrocytic proliferation (*astrogliosis*). Secondary enlargement of the ventricle can be observed in postnatal MRI in case of the integration of a cystic lesion into the adjacent ventricle (Barkovich and Raybaud 2012, Ch.4).
It was proposed that injury during neuronal migration leads to *schizencephaly*, a cortical dysplasia in which gray matter is found along the cerebral cleft, while injury occurring after termination of neuronal migration may lead to a *porencephalic cyst*. Since occurring in white matter, this type of injury does not show gray matter alignement (Harada et al. 2017).
In a patient harboring a *COL4A1* mutation, schizencephaly was found at the 35th GW, after detection of a brain injury at the 19th gestational week (GW) in the same brain region on fetal ultrasound (Khalid et al. 2018). Khalid et al. observed that the detected brain injury occurred during the period of neuronal migration, and subsequently developed into schizencephaly. According to the authors, this finding supports the hypothesis that schizencephaly can be the consequence of a pathologic vascular event occurred during the stage of neuronal migration (Khalid et al. 2018). Further cases reinforcing this hypothesis are evaluated in Table *11* of our study.

1.2.7.3 Brain maturity and injury localisation

The patterns of hypoxic-ischemic brain injury change with gestational age, since depending on maturity stage, different brain regions show different degrees of vulnerability to hypoperfusion and hypoxia (Barkovich and Raybaud 2012, Ch. 4). Moreover, duration of the injury and severity of reduction in cerebral blood flow affect the resulting injury patterns (Barkovich and Raybaud 2012, Ch. 4). In preterm infants or fetuses, periventricular and deep white matter are injured during hypoperfusion, leading to periventricular leukomalacia. Germinal matrix[11] hemorrhage leading to

[11] Germinal matrix: Neurons and in later stages, glial cells are generated in germinal matrix, with main activity between the 8th and 28th gestational week. A germinal matrix is found in the ventricular wall and in the external granular layer of the cerebellum is a germinal zone. In these areas vessels show thin walls and high sensitivity to variations in oxygen supply and blood flow (Barkovich and Raybaud 2012).

peri- and intraventricular hemorrhage (IVH) or cerebellar hemorrhage is typical for immature brains (Barkovich and Raybaud 2012, Ch.4).
In term neonates, more often cortical and subcortical areas are injured. In context of bilateral periventricular hemorrhagic infarction, or if a familiar history is known, e.g. for porencephaly, and in case of IVH in the term neonate, searching for *COL4A1* mutations has been suggested (Barkovich and Raybaud 2012, Ch.4). In a cohort of 41 preterm neonates with IVH screened for *COL4A1* mutations, one maternal inherited *COL4A1* mutation was found in dizygotic twins (Bilguvar et al. 2009). In another study considering association of intracranial hemorrhage (ICH) with *COL4A1* mutations, four preterm neonates with ICH were tested for *COL4A1/-2* mutation and no mutation was found. The authors speculated that ICH does not seem to be related to *COL4A1* mutation if no porencephaly or recurrent hemorrhages are found (Kutuk et al. 2014).

1.3 Epilepsy: Fundamentals

Epilepsy is the most common chronic and severe neurologic disease in the world, with a cumulative lifetime incidence of about 3 % and incidence peaks in childhood and the elderly (Kobow and Blümcke 2017; Berkovic et al. 2006; Hauser et al. 1993). Epilepsy itself is defined as the condition of recurrent, unprovoked seizures, while seizures represent a paroxysmal, hypersynchronous and excessive discharge of neurons in the brain. Seizures lead to an alteration of neurologic function (Stafstrom and Carmant 2015).

1.3.1 Etiology

Brain lesions, inflammation and genetic causes may lead to an imbalance in neuron excitation and inhibition, and thus to epilepsy. Intracellular pathways as well as ion channels, synapses or neuronal networks may be impaired. Mostly, several factors together play a role in this condition (Neubauer et al. 2014).

In 1981 the International Classification for Epilepsy distinguished symptomatic from idiopathic and cryptogenic epilepsy. Since 2010, the International League Against Epilepsy (ILAE) recommended that these three entities should instead be defined as following: “structural/metabolic” epilepsy, “genetic” epilepsy or epilepsy of “unknown cause” respectively (Berg et al. 2010).

According to estimations, in approximatively 20-30% of epilepsy an external factor such as stroke or trauma is underlying (Hildebrand et al. 2013). In the remaining epilepsy cases, genetic factors are supposed to play a major role (Hildebrand et al. 2013).

Most genetic epilepsies are not inherited in a Mendelian way, and genes act together with environmental or traumatic factors triggering epilepsy (Neubauer et al. 2014, Ch.2). Though in some cases, monogenic inherited forms of epilepsy are found (Neubauer et al. 2014, Ch.15).

Interestingly, in structural epilepsy, lesions can be of genetic origin (Berg et al. 2010). An example for a genetic condition related to structural epilepsy is tuberous sclerosis complex (Neubauer et al. 2014, Ch. 2; Berg et al. 2010).

As shown in our study, *COL4A1/-2* related epilepsy is a genetic condition associated with structural changes.

1.3.2 Drug-resistant epilepsy

First-line therapy of epilepsy consists in medical treatment with antiepileptic drugs (AED). Most childhood seizures can be successfully controlled with AEDs (Tolaymat et al. 2015).

Drug-resistant epilepsy is defined as “failure of adequate trials of two tolerated, appropriately chosen and used antiepileptic drug schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom” (Kwan et al. 2010).

According to a long-term study on childhood-onset epilepsy, about 30% of the patients did not achieve remission, including 19% with drug-resistant epilepsy from the start to the end of follow-up (average 37 years) (Sillanpää and Schmidt 2006).

In about one third of all epilepsy patients, failure of remission after two AED regimes is to be expected and evaluation for epilepsy surgery is needed (Ramey et al. 2013).

A meta-analysis showed that in drug-resistant epilepsy, epilepsy surgery achieved higher seizure-freedom rates, less need for AEDs, as well as better quality of life than treatment with AEDs (Liu et al. 2018).

1.4 Epilepsy surgery: Fundamentals

2001, a randomized control trial showed that in focal temporal lobe epilepsy, surgical treatment is superior to AED treatment (Wiebe et al. 2001). Consensus guidelines recommend neurosurgical evaluation in drug-resistant focal epilepsies also in childhood. Especially in catastrophic epilepsy, early surgical treatment should be achieved to prevent developmental delay or regression (Cross et

al. 2006). Since the epileptic discharges lead to impairment in function and development of healthy brain areas, better developmental outcomes are expected after resective surgery. This was shown in the surgical treatment of drug refractory epileptic spasms (Asarnow et al. 1997). Resective surgery also showed reduced risk of infant-onset epilepsy induced encephalopathy, in which uncontrolled seizures lead to neurologic disability (Jonas et al. 2005). Thus, particularly childhood focal drug-resistant epilepsies and drug-resistant epilepsies with developmental regression are important indications for presurgical evaluation in children (Neubauer et al. 2014, Ch.20). Examinations required in presurgical evaluation to assess surgical candidacy are introduced in section 1.4.1.
The main goal of epilepsy surgery is to achieve seizure freedom, but in children also a better developmental trajectory can be pursued (Reinholdson et al. 2015). After resective epilepsy surgery in children under four years, a majority showed seizure freedom or a worthwhile reduction of seizure frequency at the follow-up five or ten years after surgery (Reinholdson et al. 2015).
Procedures for different types of surgery are described in section 1.4.2.
Core principle in epilepsy surgery consists in the identification and surgical resection or disconnection of the epileptogenic zone (EZ). The EZ is defined as the brain area, that is necessary and sufficient for epileptic activity to begin (Engel 1996). Coevaluation of semiology, neuroimaging and EEG recordings is required for defining the EZ and is performed during presurgical evaluation. Anyway, the EZ is a theoretical concept, since also a potential EZ exists, which can show epileptogenic activity after surgery and may not be detected before surgery (Rosenow 2001).
One important limiting factor in epilepsy surgery is the *eloquent cortex*, which is the cortex with an attributed function that must be preserved while resecting or disconnecting the EZ to avoid permanent deficits after surgery (Rosenow 2001). It is necessary to weigh up risks and benefits of a surgical procedure within presurgical evaluation. Post-surgical deficits and complications are described in Ch. 1.4.3.

1.4.1 Presurgical Evaluation

An interdisciplinary case conference considering clinical, neuropsychological, EEG and MRI findings is needed for presurgical evaluation (Arzimanoglou et al. 2016, Ch. 27).

1.4.1.1 Semiology

Semiology, i.e. the clinical analysis of seizure signs, can help to identify candidates showing focal onset seizures, which are generated in one brain hemisphere. Nevertheless, seizure features may be very subtle in childhood. Moreover, the same brain region can lead to different seizure types depending on developmental age (Arzimanoglou et al. 2016, Ch. 1)

1.4.1.2 EEG

Surface EEG discharges recorded ictally (during the seizure) constitute the only proof of an epileptic event (Arzimanoglou et al. 2016, Ch. 2). Interictal recordings showing focal polymorphic slowing, burst suppression or attenuation are hints for an underlying focal structural lesion (Noh et al. 2013). Interictal paroxysmal discharges, such as spikes/sharp waves, or focal fast discharges can help in localising the EZ. During wakefulness and rapid eye movement sleep, spikes have a greater localizing value. Also continuous epileptiform discharges can be helpful clues for the identification of the EZ (Arzimanoglou et al. 2016, Ch. 2).
Scalp video-EEG is the main investigation element in presurgical evaluation of children with drug-resistant epilepsy (Jayakar et al. 2014). Nevertheless, surface EEG shows limitations: focal seizures can be present in epilepsy syndromes which are not suitable for surgery (Arzimanoglou et al. 2016, Ch. 2). Non-congruence of EEG findings and structural abnormalities may be due to limitations of scalp-EEG and is particularly observed in malformations of cortical development, which show a complex anatomical distribution with “modulation of epileptiform discharges before recording on

the surface" (Sisodiya 2000). Thus, additional techniques such as MR neuroimaging, as well as possibly intracranial EEG are needed for a more extensive evaluation.
Intracranial EEG (iEEG) can be considered the gold standard to identify the EZ. IEEG with subdural electrodes, embedded in grids or strips, or with combined depth electrodes, can be performed intraoperatively or extraoperatively (Arzimanoglou et al. 2016, Ch.6). If performed intraoperatively, iEEG helps in guiding resection and identification of eloquent cortex and can be also defined as intraoperative "electrocorticography" (ECoG) (Arzimanoglou et al. 2016, Ch.6). If performed extraoperatively, following surgical implantation of electrodes, iEEG can provide information regarding eloquent cortical areas, onset and propagation of interictal and ictal epileptic discharges in order to define the surgical approach (Arzimanoglou et al. 2016, Ch.6).

1.4.1.3 Neuroimaging

Neuroimaging is needed in presurgical evaluation for identifying structural lesions and eloquent cortex.
High resolution structural MR-imaging (3 Tesla or 1.5 Tesla) is to be performed according to standardised epilepsy protocols. Depending on age, different sequences may be studied. In infants, sagittal, axial and coronal T2 sequences are most helpful. 3D T1 weighted and T2 fluid attenuated inversion recovery (FLAIR), as well as oblique coronal high resolution T2 perpendicular to the hippocampal formation should also be obtained.
MRI can identify structural lesions, such as malformations of cortical development (MCDs) including focal cortical dysplasia (FCD) and heterotopias, vascular or inflammatory lesions, and temporal sclerosis or tumors. Markers for FCD are e.g. gray matter signal changes, changes in cortical thickness, blurring of gray-white barrier and white matter signal changes with or without transcortical changes (transmantle sign) (Colombo et al. 2009).
However, lesions identified on MRI are not necessarily epileptogenic, and thus EEG and possibly intracranial EEG are needed to confirm epileptogenicity (Rosenow 2001). In case of multifocal abnormalities, functional imaging such as positron-emission tomography (PET), single photon emission computed tomography (SPECT) or source imaging, e.g. EEG event related blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI) can be performed.
This, and several other limitations of neuroimaging, including level of expertise of the analysing neuroradiologist, or age-dependent changes in the patients, such as myelination progressing in infants, should be considered. If myelination is not completed, white matter areas can appear isointense to the cortex (Colombo et al. 2009; Arzimanoglou et al. 2016 Ch.3). Even high resolution-MRI may fail to delineate the exact extension of structural lesions such as MCD. Here, only atrophia of hypoplasia or the lobe may give the hint for MCD. In cases without histopathological examinations, MCDs could be easily overlooked and considered the consequence of ischemic damage (Krsek et al. 2010).
In *MRI negative*-cases, in which no potential epileptogenic lesion is found in conventional clinical MRI, surgical treatment is not excluded, even if postoperative outcome is expected to be reduced compared to MRI-positive patients. In case of MRI-negative resections, histopathology mostly reveals a specific epileptogenic correlate. Some authors recommend to define the epilepsy as *non-lesional,* if the resected specimens show normal or only unspecific histopathology (Arzimanoglou et al. 2016, Ch. 27). However, it must be considered that the wrong region may have been resected, leading to unspecific findings in such cases.
In summary, neuroimaging should be interpreted in the context of clinical and functional data.

1.4.2 Procedures in childhood epilepsy surgery

Depending on the location of the EZ, on epilepsy etiology, on eloquent cortex proximal to the EZ and on the performing surgeon different techniques may be chosen. Assisting devices such as a microscope, neuronavigation techniques, ECoG and intraoperative monitoring are used.
In the following, procedures performed in patients of this study are presented.

Lesionectomy: An adequate exposure of the area is needed. After a skin incision, a hole and a bone flap is made with a high-speed drill. The dura is opened and suspended. Aided by anatomic orientation, neuronavigation, intraoperative ultrasound and ECoG, the lesion is identified and a subpial resection is performed. An *en-bloc* resection of the lesion is performed. If preresective ECoG was performed, also after resection ECoG is performed. Dura is closed and the bone flap is fixed (Arzimanoglou et al. 2016, Ch. 29).
In temporal lobe epilepsy, the surgical approach depends on the etiology of epilepsy, the extent of EZ, the involvement of mesial structures and lateralization. Three main types of surgery can be distinguished: *anterior temporal lobe resection*, selective mesial resection (*amygdalohippocampectomy*) and *neocortical lesionectomy* (Arzimanoglou et al. 2016, Ch. 30). The standard anterior temporal lobectomy is usually combined with amygdalohippocampectomy (Arzimanoglou et al. 2016, Ch. 30). Recent approaches such as *selective amygdalohippocampectomy* or keyhole procedures with amygdalohippocampectomy strive to conserve as much neocortex as possible (Ramey et al. 2013; Arzimanoglou et al. 2016, Ch.30).
In case of focal intractable epilepsy caused by a condition involving an entire cerebral hemisphere, hemispherectomy or hemispherotomy are frequently performed (Cross et al. 2006; Kim et al. 2018). *Hemispherotomy* is a less invasive procedure than hemispherectomy, since a disconnection, but no resection of the hemisphere is performed. Thus, postoperative complications due to cerebral cavity formation occur less frequently. The lesional hemisphere remains vascularized but is disconnected from the contralateral hemisphere and from the ipsilateral thalamostriatal region. In hemispherotomy, *corpus callosotomy* and transection of the fornix are always performed (Arzimanoglou et al. 2016, Ch. 33). Corpus callosum is the major cortical interhemispheric white matter tract and connects homotopic regions of the opposite hemispheres, coordinating interhemispheric modulation and bilateral activities. Disconnection prevents spreading of epileptic potentials and seizure generalisation (Arzimanoglou et al. 2016, Ch. 38). In hemispherotomy, afferent fibers and thus the input to the anterior commissure are disconnected. Basal ganglia are disconnected from the cortical layers of the temporal lobe, the frontal lobe and the insula. In addition, the disruption of the long descending tracts is crucial in order to increase the likelyhood of seizure freedom.
The same technique can be applied to the posterior part of one hemisphere and is then called *posterior multilobar disconnection* (Arzimanoglou et al. 2016, Ch. 33).
Palliative procedures: in some patients, epilepsy surgery is not performed with a curative, but with a palliative perspective. This means, seizure freedom is not expected after surgery. Procedures include *corpus callosotomy* or vagal nerve stimulation (Schuele and Lüders 2008).

1.4.3 Complications in epilepsy surgery

Surgical, neurological and neuropsychological complications can be observed after epilepsy surgery. Surgical complications include hydrocephalus, coagulopathy, infections, aseptic meningitis and cerebral infarction.
Complications after temporal lobe surgery reviewed by Georgiadis et al. in 2013 include visual field deficit, hemiparesis, dysphasia/aphasia, cranial nerve palsy and neuropsychological morbidity, such as memory decline and depression. Cumulative morbidity amounts to 0-9% in the pediatric patients, mortality approaches zero in this review (Georgiadis et al. 2013).
After hemispherotomy, hemianopia is expected in all patients. As reviewed by Kim at al. in 2018, transient aggravation of hemiparesis is observed in most patients after surgery, but no severe aggravation of hemiparesis persists in the long term. According to this review, hand function is in most cases more severely impaired than walking. In about 10% of the cases a second-look surgery is needed, e.g. in case of seizure persistence and MRI showing incomplete disconnection. Perioperative

mortality is higher than 1% (Kim et al. 2018).
Thus, epilepsy surgery needs to be discussed in interdisciplinary case conferences to outweigh advantages and complications in each individual case.

1.4.4 Evaluating outcome

Many factors can influence outcome after epilepsy surgery, such as localisation of the EZ, epilepsy etiology, operative procedure, age of the patient, as well as proximity of the eloquent cortex to the EZ.
Several parameters can be considered when evaluating outcome, such as seizure freedom, cognitive development and quality of life. Avoidance of sudden death in epilepsy (SUDEP) or improvement of catastrophic epilepsy may be difficult to evaluate.
According to the most frequently used classification, the Engel Classification, seizure-freedom can be classified in four grades. See Table *1* for Engel Classification (Engel 1993) and corresponding ILAE classification.

Engel Classification of Postoperative Outcome	**ILAE-Classification**
Class I. Free from disabling seizures A. Completely seizure free since surgery B. Nondisabling simple partial seizures only since surgery C. Some disabling seizures after surgery, but free from disabling seizures for at least 2 years D. Generalised convulsions with AED discontinuation only	**Class 1.** Completely seizure free; no auras **Class 1a**. Completely seizure free since surgery; no auras **Class 2.** Only auras; no other seizures
Class II. Rare disabling seizures ("almost seizure free") A. Initially free from disabling seizures, but still rare seizures B. Rare disabling seizures since surgery C. Occasional disabling seizures since surgery, but rare seizures for the last 2 years D. Nocturnal seizures only	**Class 3.** One to three seizure days per year; +/- auras
Class III. Worthwhile improvement A. Worthwhile seizure reduction B. Prolonged seizure-free intervals amounting to >50% of follow-up period, but not <2 years	**Class 4.** Four seizure days per year to 50% reduction of baseline seizure days; +/-auras
Class IV. No worthwhile improvement A. Significant seizure reduction B. No appreciable change C. Seizures worse	**Class 5.** Less than 50% reduction of baseline seizure days to 100% increase of baseline seizure days; +/- auras **Class 6.** More than 100% increase of baseline seizure days; +/- auras

Table 1. Engel and ILAE Classification of postoperative seizure outcome.
From: (J. Engel, G.D. Cascino, P.C.V. Ness, T.B. Rasmussen, L.M. Ojemann 1993; Wieser et al. 2001; Durnford et al. 2011)

1.4.4.1 Outcome in temporal lobectomy and hemispherotomy

Outcome after temporal lobectomy in a randomized controlled trial about patients older than 16 years was very good, with 58% seizure freedom rates and less seizures impairing awareness than in the AED-treatment group (Wiebe et al. 2001). In a retrospective study comparing outcome after temporal lobectomy, children were found to have a good, but slightly smaller seizure freedom rate (63%) than adults (72%) (Lee et al. 2010).
In a recent meta-analysis about 1528 patients among all age groups who received hemispheric surgery (hemispherectomy or hemispherotomy) the authors showed that 73% of the patients were seizure free after surgery. Moreover, the authors could define several predictors for outcome: developmental disorders, generalised seizures, nonlateralization in EEG, and contralateral MRI abnormalities were shown to be relative risk factors of seizure recurrence (Hu et al. 2016)
A long-term study about hemispherotomy in childhood showed 74% rates of seizure freedom, and about 12% of the cases were classified as Engel II, and 14% as Engel III-IV. Variation according to epilepsy etiology was not shown to be significant (Delalande et al. 2007).

Long-term outcome in epilepsy of infancy for all surgical procedures was shown to be favorable, with 50% of seizure-free children and an additional 31% of the cases with a >75% reduction in seizure frequency (Reinholdson et al. 2015).

1.4.4.2 Outcome in surgery for genetic epilepsy

Surgical outcome in genetic refractory epilepsy varies depending on genetic cause (Stevelink et al. 2018). In the systematic review of Stevelink et al. success of surgery was assessed in a total of 82 patients with different genetic background. The main results of this review are described in the following: seizure freedom (SF) after surgery was achieved in 2/14 patients (14%) with germline mutations involving channel function and synaptic transmission (*SCN1A, SCN1B, CNTNAP2, STXBP1*). Significantly higher rate of SF was achieved in patients with mutations affecting the mTOR pathway: among patients with somatic or mosaic mutations in *PIK3CA, AKT3* or mTOR, 15/18 individuals (83%) were seizure free, while 7/12 (58%) patients with germline mutations in the mTOR pathway (in *DEPDC5, PTEN, NPRL2, NPRL3*) showed SF. If the epilepsy was caused by other genetic causes (neurofibromatosis type 1, microdeletions, fragile X syndrome, mitochondrial mutations), SF was observed in 24/38 (63%) of the patients. Surgery was less effective in MRI-negative epilepsy cases (SF in 33%) than in MRI-positive ones (SF in 63%). Even when only the MRI-positive cases were compared, lower SF rate was observed in germline mutations leading to channelopathies and disorders of the synaptic pathway (1/9) than in cases with disorders of the mTOR pathway (germline mutations: 5/8, somatic or mosaic mutations: 15/18). Stevelink et al. suggested that more localized malformations may arise in patients with mutations of the mTOR pathway, leading to higher success rate of surgery in comparison to patients with disorders of the synaptic pathway or channelopathies. Statistical analysis of differences in SF depending on surgical procedure or on histological findings was not provided. Interestingly, Stevelink et al. observed that among 12 MRI-negative patients (with mutations in *SCN1A*, *SCN1B*, *CNTNAP2*, *STXBP1*, *DEPDC5* or *NPRL2*, microdeletions in 16p13.11, or neurofibromatosis type 1), resected tissue of 5 patients showed features of malformations of cortical development (FCD Ia, IIa or not further specified); in 5/12 patients no abnormalities, in 1/12 hippocampal sclerosis and in 1/12 hamartoma were detected In histological analysis. In the mentioned study, *COL4A1* or *-2* mutation related epilepsy was not considered (Stevelink et al. 2018).

1.4.4.3 Outcome in epilepsy surgery for MCD

In epilepsy surgery for malformations of cortical development (MCD) varying seizure-freedom rates were found in different studies.

About 40% of all cases in which MCDs were histologically found, showed seizure freedom after surgery in a two-year follow up (Sisodiya 2000). In a cohort of 200 pediatric patients, seizure freedom in MCD was achieved in 54% of the cases. In this study, high resolution MRI and intracranial EEG had been used to determine the extension of MCD (Krsek et al. 2010). A meta-analysis found following factors to be associated with higher rates of postoperative seizure control in management of focal cortical dysplasia (FCD): partial seizures, a temporal location, MRI-detectable lesion, FCD Type II (according to Palmini classification) and a complete anatomical-electrophysiological resection of the lesion (Rowland et al. 2012).

Among 60 patients with histologically proven MCDs, seizure-free outcome was reached in around 80% of the patients at last follow-up (average follow-up 5.0 ± 3.0 years, median 4.0; range 1-14). MCDs located in the temporal lobe, hemispherotomies and postsurgical EEG free of interictal spikes were associated with favorable outcome after surgery. Small sample-size in the MCD-subgroups was considered to explain why no significant differences were detected in outcome with respect to the MCD-subgroups (Muhlebner et al. 2014).

In a recent retrospective study, a seizure freedom of about 53% was found several years after surgery in 58 children and adolescents with isolated FCD. Here, lesion on MRI and complete resection were the strongest positive predictors of favorable outcome (Choi et al. 2018).

1.5 Histological findings in epilepsy surgery with focus on MCD

In childhood epilepsy surgery, focal cortical dysplasia (FCD) is the most common histological finding, whereas in adult epilepsy surgery it is hippocampal sclerosis (Blümcke et al. 2017).
Moreover, cortical dysplasia is very common in younger children with extratemporal, multilobar epilepsy (Harvey et al. 2008).
Importantly, MCDs were detected in neuroimaging of all our patients, and in histological analysis of the specimens of the three patients of our cohort who underwent epilepsy surgery. Thus, MCD classification and pathogenetic aspects are presented in the following.
FCD is the most common malformation of cortical development (MCD) and was first classified 1971. Main findings in FCD are dyslamination of the cortex and blurring of the gray matter-white matter border (Taylor et al. 1971; Sisodiya 2000). Generally, MCDs are defined as cerebral structural abnormalities arising during gestation. Other examples for MCDs are *polymicrogyria*, in which overfolding of the cortex and small gyri are found, *hererotopias*, in which neurons are found to be misplaced, *lissencephaly*, in which no gyri are found, and *schizencephaly*, in which a cleft through the cortex is observed (Sisodiya 2000). Some authors also include tuberosis sclerosis in the spectrum of MCDs (Barkovich et al. 2005).

1.5.1 MCD: Histological classification

The histological classification for MCDs was introduced by Palmini et al. in 2004 and modified with the new consensus classification system for FCDs. Class I FCD is characterised by dyslamination and abnormal clustering of neurons. In class II, not only cortical organisation disruption, but also abnormal cells and ballon cells are found (Palmini et al. 2004).
The new consensus classification mainly distinguishes isolated FCDs (FCD Type I and II) from dysplasias associated to other lesions, such as hippocampal sclerosis, vascular malformations or tumors (FCD Type III), see Table *2* (Blümcke et al. 2011).
Mild MCDs describe heterotopic neurons in layer I (mMCD type I) or within white matter (mMCD type II) (Blümcke et al. 2009).

FCD Type I (isolated)	Focal Cortical Dysplasia with abnormal radial cortical lamination (FCD Ia)	Focal Cortical Dysplasia with abnormal tangential cortical lamination (FCD Ib)	Focal Cortical Dysplasia with abnormal radial and tangential cortical lamination (FCD Ic)	
FCD Type II (isolated)	Focal Cortical Dysplasia with dysmorphic neurons (FCD IIa)	Focal Cortical Dysplasia with dysmorphic neurons and balloon cells (FCD IIb)		
FCD Type III (associated with principal lesion)	Cortical lamination abnormalities in the temporal lobe associated with hippocampal sclerosis (FCD IIIa)	Cortical lamination abnormalities adjacent to a glial or glio-neuronal tumor (FCD IIIb)	Cortical lamination abnormalities adjacent to vascular malformation (FCD IIIc)	Cortical lamination abnormalities adjacent to any other lesion acquired during early life, e.g., trauma, ischemic injury, encephalitis (FCD IIId)
FCD Type III (not otherwise specified, NOS):	if clinically/radiologically suspected principal lesion is not available for microscopic inspection			

Table 2. Classification of focal cortical dysplasias. From (Blümcke et al. 2009, Blümcke et al. 2011)

1.5.2 MCD: Classification in neuroimaging

MCDs were classified originally depending on neuroimaging, based on a conceptual framework considering mechanism and timing of lesion formation (Barkovich et al. 1996). This classification included three main stages: I. neuronal proliferation, II. neuronal migration and III. cortical organisation. Precisely, in the first stage, stem cells proliferate to neuronal or glia cells, in the second stage neurons migrate from the periventricular germinal matrix to the cortex and in the third stage, the six-layer cortex is established together with the processes of synaptogenesis and apoptosis. Defects in maturation of neurons would lead to dysmorphic neurons, defects in neuronal migration may lead to heterotopias (see also Attachments, 9.3). Disturbances occurring in the third stage would lead to focal cortical dysplasia without balloon cells (Barkovich et al. 1996; Barkovich et al. 2012). Microcephaly could be diagnosed when gray matter in cerebrum in neuroimaging study is decreased and was categorised as a disruption occurring in neuronal proliferation stage. Pathology occurring in one stage could affect consequent developmental stages, and MCDs were classified depending on earliest stage affected (Barkovich et al. 1996). Later, the authors started to associate the different classes to particular genotypes, to consider underlying biology (Barkovich et al. 2005). In 2012 an update of the classification was published (Barkovich et al. 2012), see Table *3* and Attachments 9.1.

I.	**Malformations due to abnormal neuronal and glial proliferation or apoptosis**	Microcephaly (MCPH1 mutation), Neoplastic: cortical hamartomas due to tuberous sclerosis (TSC1), FCD Type II (with dysmorphic neurons)
II.	**Malformations due to abnormal neuronal migration**	Lissencephaly (LIS1), Congenital muscle dystrophy syndrome (POMT), Heterotopia (FLN1) II D: Malformation due to abnormal terminal neuronal migration and defects in pial limiting membrane: Walker-Warburg Syndrome secondary to *COL4A1* mutation
III.	**Malformations due to abnormal postmigrational development**	Polymicrogyria (Xq28), Schizencephaly, FCD Type I and FCD Type III (without dysmorphic neurons), mMCD, postmigrational microcephaly

Table 3. Simplified MCD Classification (after Barkovich et al. 2012).
Examples for each class and if achievable, one possible causative gene for the disorder are provided.
Note: Different genes can lead to the same phenotype. Severity changes in the same genetic mutation as well as somatic mosaicism can lead to different phenotypes. For the complete classification, see Attachments, Ch.9.1.

Interestingly, the mutation in *COL4A1* gene is listed in this classification as causative gene for terminal neuronal migration disorder associated with pial limiting membrane defects in *COL4A1*-associated Walker-Warburg-Syndrome/Muscle-Eye-Brain (MEB) disease. MEB disease is characterised by defects in neuronal migration, ocular dysgenesis and congenital muscular dystrophy (Barkovich et al. 2012; Labelle-Dumais et al. 2011), see also page 4-5 in Attachments 9.1.

While MCD-diagnosis based on neuroimaging enables analysis of the whole brain of any patient, but shows limited resolution, histological examination and classification can offer deep insights in the pathology of the resected areas. However, histological evaluation is based on an invasive procedure and depends solely on resected areals. For the diagnosis of some types of MCDs, such as dysplastic neuronal cells, only histological examination is possible up to now.

1.5.3 Etiopathology in MCD

The timing and pathogenesis of MCDs is not clearly understood. Some authors stated that mild forms of MCD may originate near birth, while severe forms, such as dysmorphic neurons may originate in the second fetal trimester (Cepeda et al. 2006).
Environmental and genetic factors play a role in etiopathology of MCD. MCDs can originate during prenatal/perinatal brain injury, such as in case of asphyxia or stroke. Among 200 patients with histologically proven MCDs/FCDs, in 12.5% pre- or perinatal brain injury was found (Krsek et al. 2010). Krsek et al. pointed out that pre-/perinatal events were related to mMCD and FCD Type I, which were defined as “milder” than FCD type II. The authors supported the hypothesis that

histologically severe malformations (such as FCD Type II) may be caused by events during the second or early third trimester of gestation, while perinatal events might lead to milder forms of cortical malformations (Krsek et al. 2010).
Even later injuries can lead to MCDs, as shown in post-mortem examination of one 7-year- and one 9-year-old epilepsy patient who had survived injury due to shaken-infant-syndrome occurred at 11 days and 3 months of age respectively (Marín-Padilla et al. 2002). The authors proposed that the post-injury reorganization of the surviving undamaged or only partially damaged cortex may influence the neurological maturation more significantly than the encephaloclastic encephalopathies (such as porencephaly or multicystic encephalomalacia) resulting from the original injury (Marín-Padilla et al. 2002).
Moreover, impairment of vascular system has been discussed as cause of MCD (Spreafico et al. 1998b).
Furthermore, MCDs can originate depending on genetic mutations. A developmental and genetic classification for MCDs was proposed by Barkovich et al. in 2012 (see Table *3* and Chapter 9.1). Germ-line mutations need to be distinguished from somatic (postzygotic) mutations, that can lead to a *mosaicism.* In the last case, only several areas in the brain are affected (Barkovich et al. 2005).

1.5.4 Epileptogenesis in MCD

MCDs may lead to drug-resistant epilepsy, developmental delay, neonatal encephalopathy and variable levels of motor and cognitive dysfunction (Barkovich et al. 2005, Barkovich et al. 2015).
In the discussion of epileptogenesis in MCDs, a decrease in GABA-ergic interneurons and increased excitatory network in FCD was postulated (Spreafico et al. 1998a). In a immunochemical analysis of tissue, changed excitatory and inhibitory circuits in relationship to adjacent cortex were found in FCD (Alonso-Nanclares et al. 2005).
The electrophysiological behaviour of neurons from pediatric cortical dysplasia tissue was analysed in vitro: the authors observed that dysmature pyramidal neurons and cytomegalic interneurons with abnormal electrophysiologic properties show “proepileptic” features (Cepeda et al. 2006). Cepeda et al. presented the “dysmature cerebral developmental hypothesis” stating that interaction of dysmature cells with normal postnatal cells produce seizures. Moreover, they proposed that the propagation of epileptic potentials from cortical dysplastic tissue into distant cortical structures may play a role in generalized seizures in infants and in epilepsy syndromes such as infantile spasms (Cepeda et al. 2006).
In summary, epileptogenesis in MCD is still not profoundly understood and is a topic of ongoing research.
Importantly, MCDs and normal functioning brain areas may overlap, thus constituting an important biologic limitation for epilepsy surgery (Sisodiya 2000).

2 Patients and methods

In this explorative and retrospective study, a literature review and the evaluation of patients´ clinical history were performed.

2.1 Patients

A retrospective review of clinical history and findings in *COL4A1/2* related epilepsy patients with and without surgical procedure was performed. The study was in agreement with the guidelines of the Declaration of Helsinki and informed consent of the patients or of their parents was obtained.

Inclusion criteria for the patients were: mutation in *COL4A1* or *-2* gene, drug-resistant epilepsy, presurgical evaluation at Epilepsy Center for Children and Adolescents Vogtareuth, neuroimaging results available, age under 18. In addition to the mentioned criteria, further inclusion criteria for patients who underwent epilepsy surgery were: epilepsy surgery at Epilepsy Center for Children and Adolescents Vogtareuth, histologic material and outcome at minimum six months after surgery available. In the investigation time up to March 2019, three patients who underwent epilepsy surgery (patients 1-3, see *Table 5*) and six patients who did not undergo epilepsy surgery (patients 4-9, see Table *6*) matching the inclusion criteria were identified and included in the study.

Clinical history of the patients was evaluated regarding the aspects: gestation and delivery, family history, epilepsy history and semiology, neuroimaging and EEG results (patients 1-9) as well as surgical procedure (patients 1-3).
Duration of surgery was in patient 1: 4 hours (h) 10 minutes (min) (first intervention), 2 h 59 min (second intervention); in patient 2: 5 h 10 min and in patient 3: 2 h 33 min. Surgery was performed under general anaesthesia with continuous application of propofol, remifentanyl and sevoflouran, supplemented by fentanyl single shots if needed.
Video-EEG monitoring and high-resolution MRI were performed before surgery. High resolution MRI including 0.6 mm 3D-T2, 1.2mm 3D FLAIR, 1mm 3D T1 mpr sequences with 1.5 Tesla was performed in the Epilepsy Center for Children and Adolescents Vogtareuth. Findings were examined by experienced pediatric neuroradiologists.
Genetic diagnosis was performed in cooperating genetic diagnostic centers.
Brain tissue specimens were obtained from resected areas and formol-fixed paraffin-embedded tissue was sent to the reference pathology center (Prof. I. Blümcke and Dr. R. Coras, Department of Neuropathology, the Neuropathological Reference Center for Epilepsy Surgery at the University Hospital Erlangen). Diagnosis was made in this center according to the currently used classification systems.

2.2 Review of literature

Review of literature was performed up to February 2019 using PubMed searching engine.
PubMed search criteria included: *COL4A1, COL4A2, COL4A1/COL4*A2 and epilepsy, *COL4A1/2* and epilepsy surgery, *COL4A1/2* and basement membrane, Collagen IV and basement membrane. Also further articles which were cited in articles matching primary search criteria were considered.
The articles were evaluated concerning these aspects: neuroimaging, epilepsy and epilepsy surgery, genetics, mechanisms of pathogenicity, malformations of cortical development and prenatal brain injury related to *COL4A1/2* mutation.

Three topics were presented extensively after a literature review in the investigation time up to 15th February 2019 using PubMed searching engine (see Table *9*, Table *10* and Table *11*):

1) Epilepsy surgery: three articles reporting surgery in three patients with *COL4A1* mutations were found.
2) MCD in *COL4A1/2*: PubMed search criteria included *COL4A1/-2* and malformations of cortical development/cortical dysplasias/FCD/schizencephaly/histology: ten articles about in total 32 patients were detected.
3) Documented prenatal brain injury: PubMed search criteria included *COL4A1/2* and prenatal brain injury/intrauterine stroke/intrauterine hemorrhage. Twelve articles documenting in total 17 cases were found.

3 Results

In section 3.1, patients included in our study are presented. Findings in patients 1-3 (who underwent epilepsy surgery) and those in patients 4-9 (who received conservative treatment) are highlighted in *Table 5* and *Table 6* respectively. *Table 8* summarises neuroimaging findings and underlines the detection of cortical abnormalities in all our patients.
In section 3.2, results from literature review on patients with *COL4A1/-2* mutations are presented. Three topics were examined in our literature review:
First, reports on epilepsy surgery were analysed (see *Table 9*). Secondly, reported cases of patients carrying *COL4A1/-2* mutations with MCDs were investigated (see *Table 10*) and confirmed the relevance of the finding of cortical abnormalities in our cohort, supporting the hypothesis that epilepsy in patients with *COL4A1/-2* mutations may be associated with MCDs. Thirdly, review on documented pre/perinatal brain injury in patients carrying *COL4A1/-2* mutations (see *Table 11*) helps to understand the timing and mechanism leading to CNS lesions and clinical manifestation in *COL4A1/-2* related disease.

3.1 Patients of our cohort

3.1.1 Patients who underwent epilepsy surgery

3.1.1.1 Patient 1

3.1.1.1.1 Gestation and delivery

This patient is a female and was delivered by caesarean delivery in 39th gestation week, with birth weight, length, head circumference and Appearance, Pulse, Grimace, Activity and Respiration (APGAR) score of 2550 g (3rd percentile (P)), 48 cm (8 th P), 32 cm (2nd P) and 10/10. Her postnatal adaptation was normal. There were no gestational abnormalities except for hyperemesis gravidarum.

3.1.1.1.2 Family history and genetics

The mother of the patient suffered of intracerebral bleeding at 24 years age and had a miscarriage in the tenth gestational week at 28 years age, in a gestation following the birth of the patient. The cousin of the grandmother on the mother side had an unspecified epilepsy.
Patient´s caryotype 46, XX did not show any abnormality.
The genetic mutation in this patient was first detected at nine years´age. The patient and her mother showed the heterozygote mutation in *COL4A1* gene c.3985G>A p. (Gly1329Ser) with autosomal dominant inheritance. To our knowledge, this mutation was not previously reported in literature. In genetic analysis, this mutation was classified as probably pathogenic (Class 4 in the IARC classification). This mutation represents a missense-mutation leading to substitution of the amino acid Glycin with Serin in the triple-helical region of the COL4A1 protein.
A homozygote mutation in *MTHFR* gene position c.677T with normal homocysteine levels was also detected in the patient and was interpreted as probably not pathogenic.

3.1.1.1.3 Milestones and general presentation

At age six months, the patient showed flaccid rightsided hemiparesis and left-handedness. At 16 months, the patient couldn´t sit and showed a general psychomotoric development delay and a spastic hemiparesis of the right side. At three years of age she could produce three words. She showed strabismus convergens of the right eye and frequent respiratory infections, hexadaktily of her right foot and pes planovalgus.

3.1.1.1.4 Epilepsy history

The patient showed first epileptic seizures at the age of 11 months. Semiology included changes in behaviour and paresis of the right arm. Beginning with 12 months she showed prolonged tonic-clonic seizures with right arm stiffening, cloni of the right arm and need for emergency medication.

Sometimes, gaze deviation to the right could be observed. Seizure frequency varied depending on her age. During preoperative monitoring at age 2 ½ years, she showed seizures every 2-4 weeks and had been on therapy with carbamazepine, oxcarbazepine and topiramate. Seizure freedom was achieved for several months with combination of oxcarbazepine and topiramate.

3.1.1.1.5 EEG

Video-EEG monitoring performed at two and a half years age showed interictal status on the left hemisphere (see Figure *2*) and continuous slowing parieto-occipital in the left hemisphere. Asymmetry, consisting in reduced beta activity and sleep spindles in the left hemisphere could be observed. Moreover, at the age of 2 years and 11 months, spikes and EEG seizure patterns in the left temporo-occipital region could be recorded. No clinical seizures during EEG recording were observed.

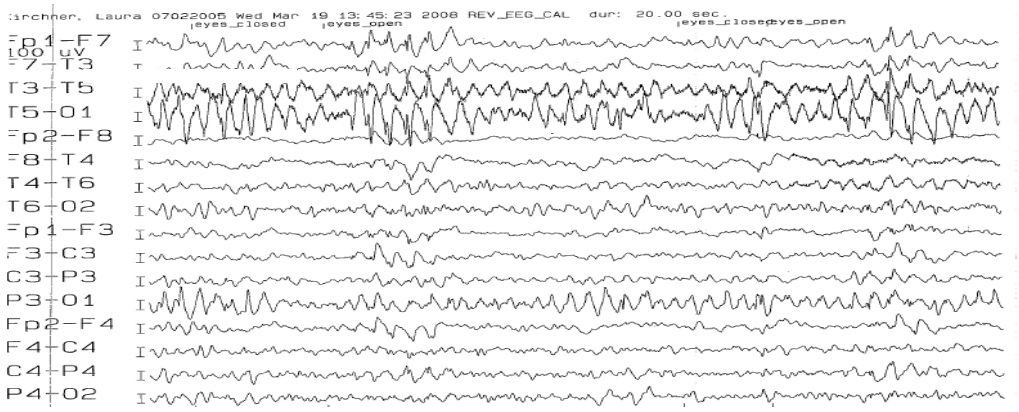
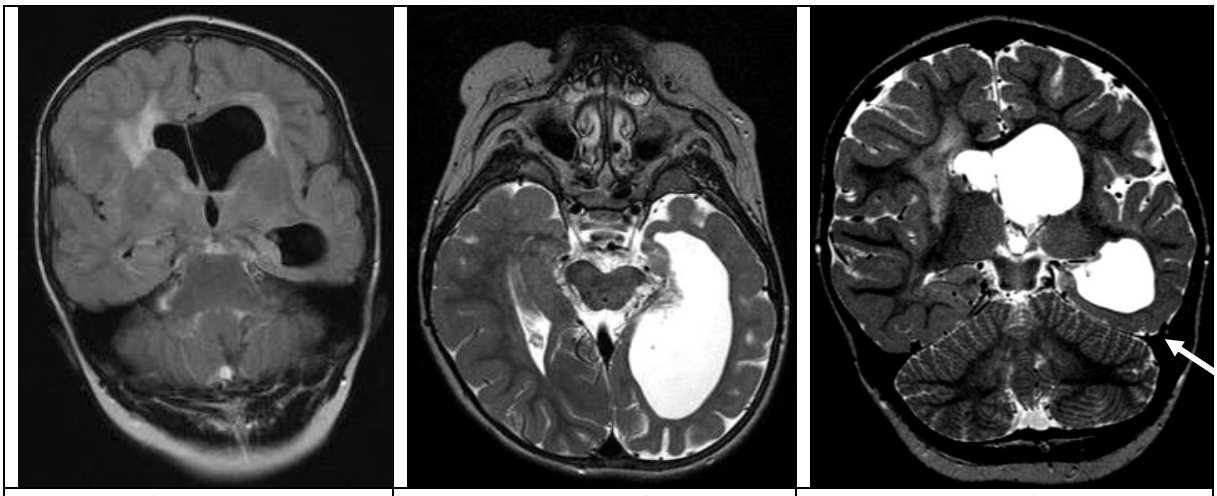


Figure 2. Preoperative EEG, Pat. 1.
Status of the left hemisphere, temporo-occipital region.

3.1.1.1.6 Neuroimaging

In a cranial MRI at ten months age, which was performed to find the cause of right-sided hemiparesis, left-sided ventriculomegaly was seen and a prenatal stroke of left middle cerebral artery was suspected. This diagnosis was then established in MRI at two years age, based on atrophy of white matter in the left hemisphere, especially in the temporal region. Later, a venous hemorrhagic infarction instead of arterial stroke was hypothetised.
MRI at two and a half years age showed ventriculomegaly of the left lateral ventricle (with a cella media Index[12] of 0.49) and reduction of cortical and white matter volume in the left hemisphere. Architecture of mesial temporooccipital cortex was pathologic, a rarefying of gyration could be observed.
Periventricular leukomalacia and deep white matter change were present in both hemispheres. Correspondingly to white matter change and consequently impaired inter-hemispheric fiber tracts, thinning of the corpus callosum could be observed. Because of white matter change in the right hemisphere, additional right-sided ischemia was hypothetised.
Disseminated defects in the cerebellum, probably corresponding to microbleeds could be observed in the susceptibility sequence. The left thalamus showed a defect on its upper part. A hypomyelination of the pyramidal tracts could be observed especially on the left side. The left hippocampus seemed to be atrophic, the left optical radiatio was reduced.
Brain skull seemed to be flattened on the right side.
Selected preoperative neuroimaging findings are shown in Figure *3* and summarised in *Table 5.*

[12] Cella media index: used to assess ventricle size compared to brain tissue. It corresponds to the ratio of biparietal diameter of skull to maximum external diameter of lateral ventricles at cella media, the central part of lateral ventricles.



FLAIR, coronal section. Bilat. PVL accentuated on the R, and corpus callosum thinning	T2 sequence, transversal section. VM L and thinning in L brain stem	T2 space 1mm, coronal section. L temporooccipital reduced corticomedullar differentiation, see absence of gyri (see arrow).

Figure 3. Preoperative MRI, Pat. 1, at age two years and six months.

3.1.1.1.7 Epilepsy surgery

Because of refractory epilepsy and the electrophysiological and neuroimaging focus on the left hemisphere, it was decided to perform resective epilepsy surgery. Status and secondary bilateral synchrony in EEG were interpreted as possibly causative for psychomotor developmental delay, and interruption of EEG status and thus better cognitive development was aimed through surgery. Hemispherotomy or resection of the central or parietal area in the left hemisphere was avoided to preserve the innervation of the right hand and speech area. A possible worsening of the right-sided hemiparesis and rightsided homonyme hemianopia were expected and discussed with the parents of the child.
Intraoperative ECoG was performed to recognise seizure patterns in the left parietal region and eventually extend resection area intraoperatively.

The 3 4/12 year old child was positioned in supine, her head layed in the Mayfield fixation device and rotated to the right. Registration to neuronavigation data was performed. Trepanation of the left side was performed over the sphenoidal bone alae. ECoG showed a status in the occipital and postcentral region. According to ECoG, resection border was established.
The temporal lobe on the left was resected in toto from the sphenoidal bone to the Labbe´sche Veins. The left hippocampus, which appeared to be atrophic, and the amygdala were resected. Then the occipital lobe and parts of the precuneus were resected. Under ECoG monitoring, EEG-Status of cingulate gyrus could be observed, so that posterior cingulate gyrus was also resected. Resected tissue was embedded for histologic analysis. A control with ECoG showed normal electrophysiological activity. The dura was closed, and craniotomy flap fixed. An external ventricular drain was positioned.

3.1.1.1.8 Neuroimaging

In neuroimaging performed at the age of three years and nine months, expected anatomical changes following temporo-parieto-occipital lobectomy and a liquid-filled cavity in the resection area were observed. See Figure *4*.

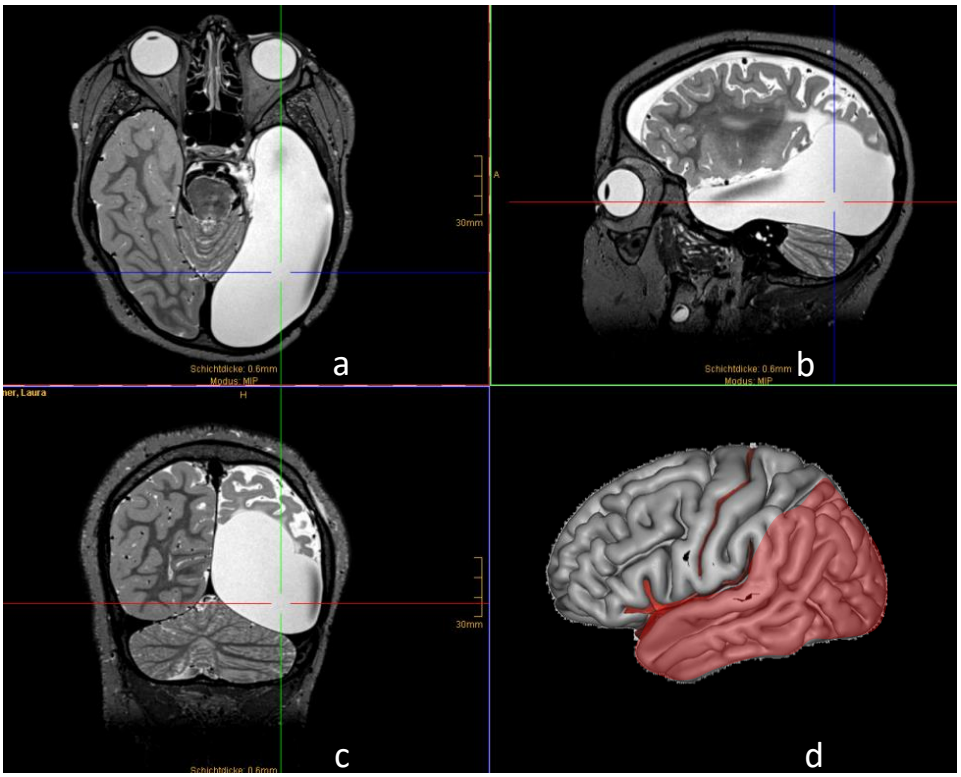


Figure 4. Postoperative MRI, Pat. 1, at age three years and nine months.
T2 sequence, a: transversal, b: sagittal and c: coronal section, d: schematic illustration of resection.

In addition, diffuse white matter hyperintensity was observed in the T2 sequence on the right side, and was first interpreted as delayed myelination, later as possible gliosis. See Figure *5*.

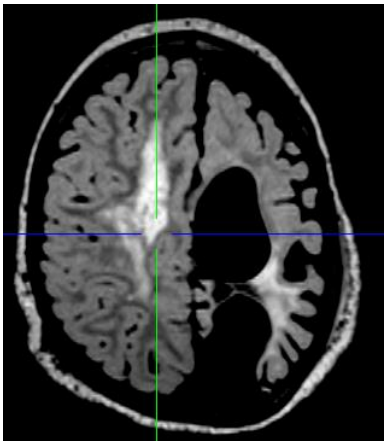


Figure 5. Rightsided white matter hyperintensity in postoperative MRI, Pat. 1, at age three years and nine months.
FLAIR, transversal section.

3.1.1.1.9 Histology

According to the ILAE criteria a focal cortical dysplasia (FCD) IIId was diagnosed, because the lesion was associated to a principal lesion (prenatal stroke). Moreover, a hippocampal sclerosis (not otherwise specified, NOS) was diagnosed. See also *Table 4*.

Temporal lobe:
A pseudocyst with ependymal dressing and gliotic change corresponding to an old damage was observed, see Figure *6*. Here, a prenatal cerebral infarction of the middle cerebral artery or a venous hemorragic infarction was proposed as causative.

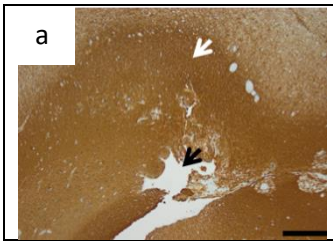
a 	Glial fibrillary acidic protein (GFAP) immunohistochemistry shows a prominent glial scar (white arrow) and parenchymal destruction (black arrow), which macroscopically appeared as a pseudocyst, corresponding to the remnants of an old infarction. Scale bar in a: 200µm, applies also for b-e.

Figure 6. Histology, Pat.1: Pseudocyst.

Focal cortical dysplasia was diagnosed based on the findings of reduced cell density in layer II and III and neuronal cell clusters, and microcolumnar organization. See Figure *7*. Since the dysplasia was associated to pseudocystic defect, it was classified as FCD IIId (see also Table *2*).

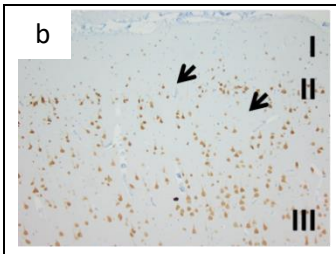
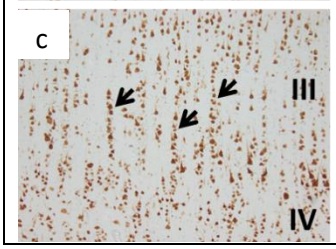
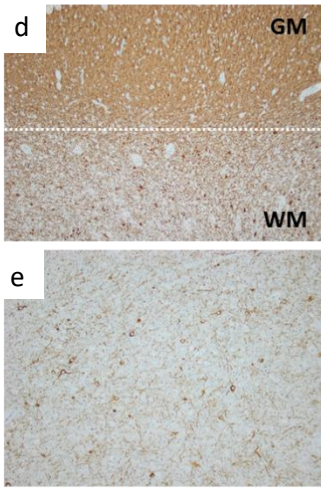
b  c 	NeuN staining shows laminar reduction of granule cells in cortical layer II and upper parts of cortical layer III, focally with gaps (black arrows in b). Other areas of the cortical ribbon with microcolumnar arrangement of neurons in cortical layers III and IV (black arrows in c). b & c: NeuN immunohistochemistry.

Figure 7. Histology, Pat. 1: Microcolumnar organisation.

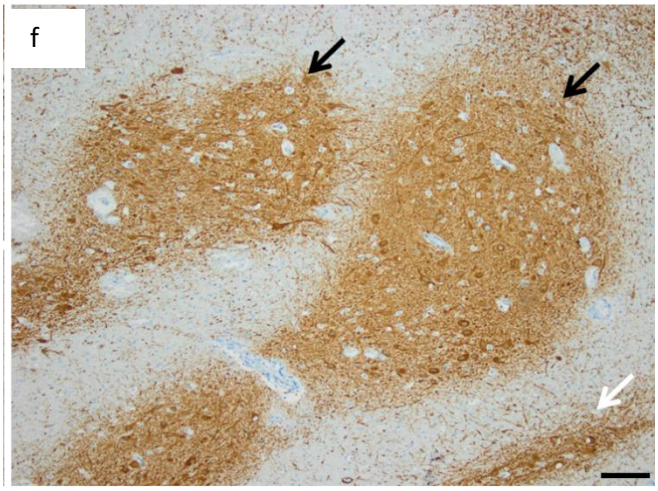
Gray matter-white matter junction was blurred. Heterotopic neurons in subjacent white matter were found. Figure *8*. No cellular dysplasias were identified.



Blurred gray matter-white matter boundary (dotted line in d) with heterotopic neurons in the subjacent white matter (d) as well as within deep white matter areas (e).
d & e: microtubule-associated protein 2 (MAP-2) immunohistochemistry.

Figure 8. Histology, Pat. 1: Blurred gray matter-white matter (GM-WM) boundary.

As Figure *9* shows, periventricular heterotopias in deep white matter were detected.

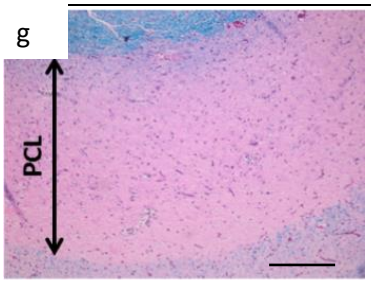


Nodular (black arrows) and lentiform (white arrow) heterotopias within the periventricular white matter.
f: MAP-2 immunohistochemistry. Scale bar: 100µm.

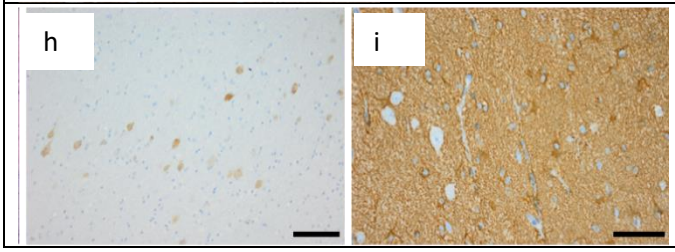
Figure 9. Histology, Pat. 1: Periventricular heterotopias.

In the temporal lobe, white matter-staining showed areas of white matter thinning. The pia mater showed fibrotic thickening with numerous blood-filled vessels.

Hippocampus: Residuals from a recent bleeding, as well as hypoxic-ischemic defects were recognized and putatively due to surgical procedure. Moreover, significant pyramidal cell loss and associated fibrillar and cellular gliosis as sign for a hippocampal sclerosis were found. See Figure *10*.



g: hematoxilin and eosin-luxol fast blue (HE-LFB) histochemistry with a fragment of the hippocampal pyramidal cell layer (PCL). Scale bar in g: 200 µm.



h**:** higher magnification and NeuN immunohistochemistry of the pyramidal cell layer of the hippocampus with reduced neuronal density. Scale bar in h: 100 µm.
i**:** Cell loss (as shown in h) is accompanied by strong gliosis. GFAP immunohistochemistry. Scale bar in i: 50 µm.

Figure 10. Histology, Pat. 1: Hippocampus sclerosis.

Occipital lobe: Fragments of a pseudocyst, corresponding to an old damage were identified. Microcolumnar organisation of neurons in the adjacent cortex was observed. The pial membrane showed a fibrotic thickening with numerous bloodfilled vessels also in the occipital lobe.

3.1.1.1.10 Postoperative course

Postoperative course was uneventful. 10 months after surgery the patient was seizure free. As expected after left occipital resection, she showed right homonyme hemianopia. She showed an impressive psychomotoric development, such as toilet training and learning of new vocabulary. Hemiparesis of the right side did not worsen significantly after operation and was classified as gross motor functions classification system (GMFCS) II, manual ability classification system (MACS) II (at age 10).

Epilepsy and outcome after the first surgical procedure

After the seizure-free interval focal seizures reappeared. Since regular, but less frequent seizures than preoperatively were observed, the outcome was classified as Engel class IIIa.
Since then, short seizure-free periods alternated with periods of recurrent seizures. The patient showed drop seizures with impaired awareness and absence seizures, and had been under therapy with carbamazepine, oxcarbazepine, topiramate, levetiracetam, zonisamide, sultiam and pregabalin.

A second surgical intervention with hemispherotomy was discussed in a multidisciplinary conference six years after first surgery but was not performed because of the relatively controlled seizures, psychological development of the patient and of the stopping of the drop seizures. The expected worsening of right hand function in case of hemispherotomy was an additional reason not to perform surgical intervention, even if a trans-cranial-magnet stimulation for evaluation of right hand innervation showed, that also the right hemisphere provided innervation to the right hand. A fMRI showed involvement of the right hemisphere in speech production, so that impairment of speech was not expected after disconnection of the left hemisphere.
In the context of the new evaluation and after accurate analysis of MRI abnormalities the genetic mutation *COL4A1* was detected.

At about 12 years age epilepsy worsened consistently and the patient presented daily recurrent drop seizures with enuresis, weekly clonic seizures and seizures with impaired awareness. Treatment with

rufinamide, clobazam and lacosamide did not lead to an improvement. The patient was severely impaired in daily life by drop seizures with need to wear a protecting helmet. Epilepsy was felt as a heavy burden in her family. Thus, a new surgical evaluation was made to consider extension of the first surgical procedure to a left-sided hemispherotomy.

EEG after the first surgical procedure
In the second presurgical evaluation, EEG status of the left hemisphere with frontocentral maximum, as well as left frontocentral subclinic seizure patterns interrupting the status could be recorded. Asymmetry, consisting in reduced sleep spindles and beta activity in the left hemisphere but also isolated spikes in the right frontal region were observed. These spikes were interpreted as irritation spikes, in consequence of the left frontocentral EEG-Status and were interpreted as a sign of reduced chance for seizure freedom after hemispherotomy.
An interruption or modification of the left-sided EEG Status could be recorded ictally and was followed by an attenuation and propagation to the right side. This corresponded to a head movement to the right, tonic distension of her arms, enuresis, smacking, dysarthria and reduced awareness.

Neuroimaging after the first surgical procedure
Severe cortical dysplasias were observed in the precentral, central and postcentral left hemisphere, as well as multifocal milder cortical changes in the right hemisphere. Right hippocampus showed reduced corticomedullar differentiation. Hypoplasia of the left brainstem was observed.

Epilepsy surgery: second procedure
The patients´ parents were informed about expected worsening of the right-sided hemiparesis, of right hand function and of rightsided hemianopia as typical complications of a left-sided hemispherotomy. Aphasia was not expected in this patient, since an atypical right-sided Wernicke areal was to be expected in the right side, even if due to impaired cooperation, functional neuroimaging could not be evaluated.
Reduced chance of seizure freedom was expected because of the additionally observed cortical changes in neuroimaging in the right hemisphere. Anyway, reduction of seizure frequency, stopping of drop seizures and improvement of cognitive function could be expected after hemispherotomy and it was decided to perform a second surgical procedure.

The thirteen years old patient was layed supine, with her head in a Mayfield fixation device. Neuronavigation registration to preoperative MRI was performed. The skull was trepanated on the middle line of the coronal suture. The dura was parasagittally opened. An extended vertical parasagittal hemispherotomy (according to the Delalande procedure (Delalande and Dorfmuller 2008)) was performed. Dura was closed and the bone flap was reinserted.

Histology findings in the second procedure
In the small resected tissue sample, horizontal dyslamination, neuronal clustering, and focal multiple blurring of the gray matter-white matter junction as well as heterotopic neurons in white matter were observed microscopically and in NeuN and MAP-2 staining. GFAP staining showed gliosis and microglial activation. Perivascular, lymphomonocytary cells could be observed.
No signs of bleeding or infarction were found. Because of the clinical diagnosis of prenatal bleeding and thus presence of a principal lesion, findings were classified as FCD Type III, not otherwise specified. See also *Table 4*.

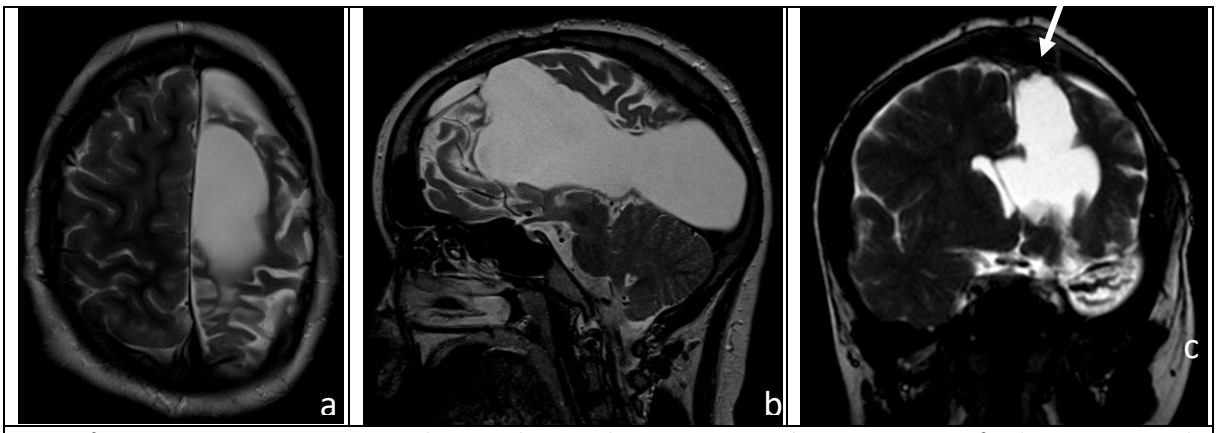
Postoperative course after the second procedure
Postoperative course was uneventful.

EEG after the second surgical procedure

EEG performed subsequently showed left-sided spikes, a continuous left-sided and generalised slowing, as well as asymmetry between the two hemispheres, with a reduction of β-waves in the left hemisphere.

Neuroimaging after the second surgical procedure

MRI following the second surgical procedure showed complete disconnection of the fibers in the left hemisphere. See Figure *11*. Diffusion abnormalities and hyperintensity near the resected area were observed in the FLAIR-sequence. A hygroma in the left frontal region was observed. Eight months after surgery, no diffusion abnormalities were found. Hemosiderin deposits lateral to the left ventricle were observed. Hyperintensities in capsula interna of the right hemisphere due to known PVL were expectedly unchanged.



T2 truefisp sequence, 5 mm. a: transversal section, b: sagittal section, c: coronal section. Imaging after lesionectomy and resection of the left temporal lobe (first procedure) and vertical hemispherotomy (second surgical procedure), arrow: surgical access to vertical hemispherotomy.

Figure 11. Postoperative MRI after second surgical procedure, Pat. 1, at age 13 years and two months.

Epilepsy and outcome after the second surgical procedure

Eight months after the second operation, the patient showed several seizures per month. Impairment of awareness was not present any more but drop seizures could occur several times per month and emergency antiepileptic drugs were needed. The patient was on treatment with oxcarbazepine, rufinamide, sultiam and nitrazepam. Sultiam was then substituted with topiramate.

Twelve months after surgery, outcome was classified as Engel IIIa.The patient showed clonic seizures of the right arm and forearm, as well as drop seizures. She had not showed seizures impairing awareneness in the previous six months. A Vagus-Nerve-Stimulator device was implanted and antiepileptic drug therapy was continued.

General development

Patient´s menarche occurred at 11 years age. At 13 years age, her body weight was observed to be on the 99th percentile. At age 14, her body weight was on the 98th percentile (BMI on the 99th percentile).

The patient showed a severe intellectual and motor disability and visited a school for disabled children. She was able to read and write some words and count in the range 0-6 and showed a speech developmental delay, unchanged to the situation before the second operation. She was not autonomous in her daily life. Hemiparesis was unchanged after the second surgical procedure and categorised into level GMFCS II, MACS II, with slightly impaired right hand function. Visual function was impaired but did not worsen after the second operation. Her behaviour was sometimes aggressive but she and her parents reported a good general condition.

3.1.1.2 Patient 2

3.1.1.2.1 Gestation and delivery

The second patient is a male and was delivered by caesarean delivery on the 37+3 gestation week. His birth weight amounted to 1920 g (<1th P), body length 44 cm (<1th P), head circumference 29.5 (<1 th P), APGAR 10/10. IUGR, caused by a probable placenta insufficiency was diagnosed. At birth, the patient showed plagiocephalus, microcephaly and mild retrognathy. In sonography, intraventricular hemorrhage of grade II° on the left side, and grade I° on the right side were detected. Toxoplasma gondii, other viruses, Rubella, Cytomegaly virus and Herpes simplex (TORCH)-serology and metabolic screening did not show pathologic results.

3.1.1.2.2 Family history and genetics

Family history on the maternal side revealed an epileptic seizure of the patient´s uncle and cheilognathopalatoschisis of the grandfather. Genetic testing in the parents was denied.
The patient caryotype was identified to be 46, XY and didn´t show abnormalities. Two heterozygote mutations in *COL4A2* were detected in his genotype: c.4084G>A (p.Gly1362Arg) and c.4684G>A (p.Asp1562Asn). Both mutations were not previously described in literature and were classified as variants of unknown significance (Class III according to the ACMG classification). The first mutation leads to a non-conservative amino acid change (Glycin to Arginin) in the THX-collagenous domain of the COL4A2 protein. Similar missense mutations in this domain were described as pathogenic in literature (Jeanne et al. 2012) and genetic analysis predicted pathogenicity.
The second mutation leads to a semi-conservative amino acid change (Aspartic acid to Asparagin) in the non-collagenous domain of COL4A2. Pathogenicity of this mutation was predicted by bioinformatic tools, but no other similar mutation was reported as pathogenic in literature.

3.1.1.2.3 Milestones and general presentation

A spontaneous Moro reflex and no reactive smiling until the fourth month was observed by the patient´s mother. Gaze fixation and reaction to visual stimuli was missing at four months age. At thirteen months the patient couldn´t sit, couldn´t turn and leftsided hemiparesis was noticed. At eighteen months the patient had no sure control of head and chest posture, was not able to sit autonomously and to crawl. A bilateral cerebral palsy (GMFCS IV-V) more pronounced on the left side was diagnosed. In addition, recurrent bronchitis was observed.
At preoperative presentation at two years four months age, the patient showed unchanged cerebral palsy, micro-, plagio- and brachycephaly. Eye pursuit movements and gaze fixation could not be performed by the patient. A preoperative examination of visual evoked potentials (VEPs) showed normal findings, so that impairment of visual perception was thought to be caused by the electrophysiologic EEG-status and encephalopathic course of the epilepsy.

3.1.1.2.4 Epilepsy history

The patient developed his first epileptic seizure at the age of seven months. Since then epilepsy semiology included tonic extension of the right leg and arm with following rightsided cloni and tonic extension of the left arm. Seizures leading to dyspnea, cyanosis and oxygen desaturation were also observed. Also generalised cloni of all limbs occurred. Received medication included levetiracetam, valproate, topiramate, oxcarbazepine and clobazam. A four-month long seizure-free period was only observed during levetiracetam treatment in his first year of life.
Shortly before the operation, the patient showed 2-3 tonic seizures of the left arm per week and 5-7 absence seizures per day.

3.1.1.2.5 EEG

EEG recordings during EEG-monitoring showed interictal continuous status bi-temporo-parietooccipital with a maximum on the right hemisphere, see Figure *12*. Continuous slowing was

recorded in awake-EEG.
EEG seizure patterns consisted of a stopping of the status, as well as bursts temporo-occipital, parieto-occipital, temporal posterior and occipital in the right hemisphere**.** In video-EEG-analysis, these seizure patterns corresponded to tonic Blitz-Nick-Salaam seizures beginning one second after EEG onset. Serially occurring tonic extension of the right leg, a head and eye deviation to the left followed the seizure patterns. A delta wave and seizure patterns correlated with head and shoulder myoclonies with open eyes. Moreover, unclear conditions with reduced awareness and fixating gaze could be observed during the monitoring, which did not correlate with EEG changes.

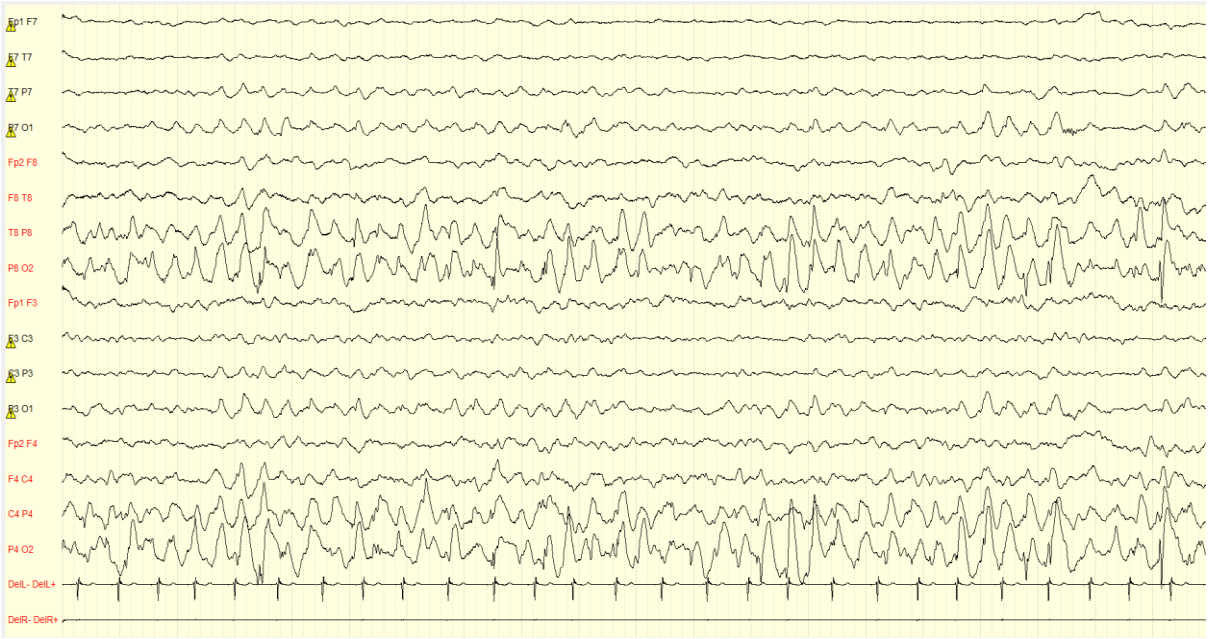


Figure 12. Preoperative EEG, Pat. 2.
Status of the right hemisphere.

3.1.1.2.6 Neuroimaging

After birth, cranial sonography showed bilateral intraventricular hemorrhages. Neuroimaging at 5 months age had showed susceptibility defects in the susceptibility weighted imaging (SWI). Here, an extended area in the right frontal and periventricular regions with bleeding residuals, which could putatively be caused by germinal matrix bleeding were observed. See Figure *13*. Moreover, in cerebellum, brain stem, subependymal and in plexus choroideus, disseminated bilateral susceptibility defects, which were thought to be due to past microbleeds were found.
MRI at 1 year and 10 months age showed bilateral periventricular leukomalacia and deep white matter change accentuated in the right hemisphere as well as thinning of corpus callosum and cortical volume reduction especially in the right occipital lobe.
Multifocal cortical abnormalities were observed particularly in the right hemisphere and were considered as the main cause of the patient´s epilepsy.
A rightsided ventriculomegaly was observed. Cella media Index corresponded to 0.39 on the right and 0.35 on the left, with a norm reference of 0.33.
Thalamus and basal ganglia seemed to be smaller in the right hemisphere compared to the left hemisphere. In addition, left hippocampus was smaller in comparison to right hippocampus, and a bihippocampal pathology was proposed. The left optic radiation showed hyperintensity changes, which were interpreted as leukomalacia.
MRI also revealed microbleeds periventricular adjacent to the anterior horn of the right and left ventricle, in the cerebellum and in the brain stem, corresponding to the earlier diagnosed defects in SWI.

In addition, a disproportion between brain skull case and facial bones was observed. Preoperative neuroimaging findings are shown in Figure *13*, Figure *14*, Figure *15*.

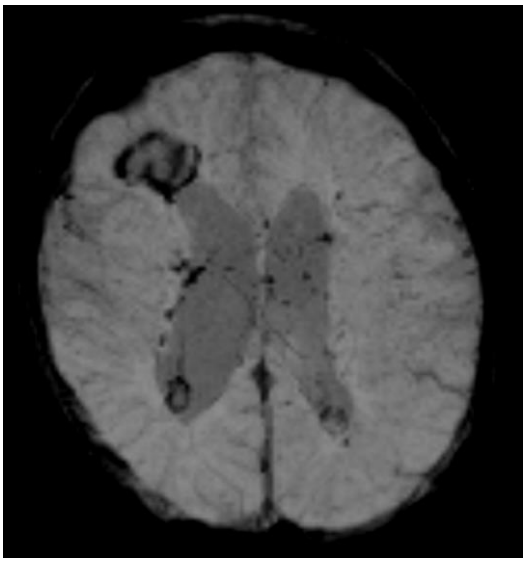


Figure 13. MRI, Pat. 2, at five months of age.
3D SWI, transversal section: bilateral multiple susceptibility defects and one major defect in the right hemisphere anterior to the ventricle, corresponding to past microbleeds and bleedings.

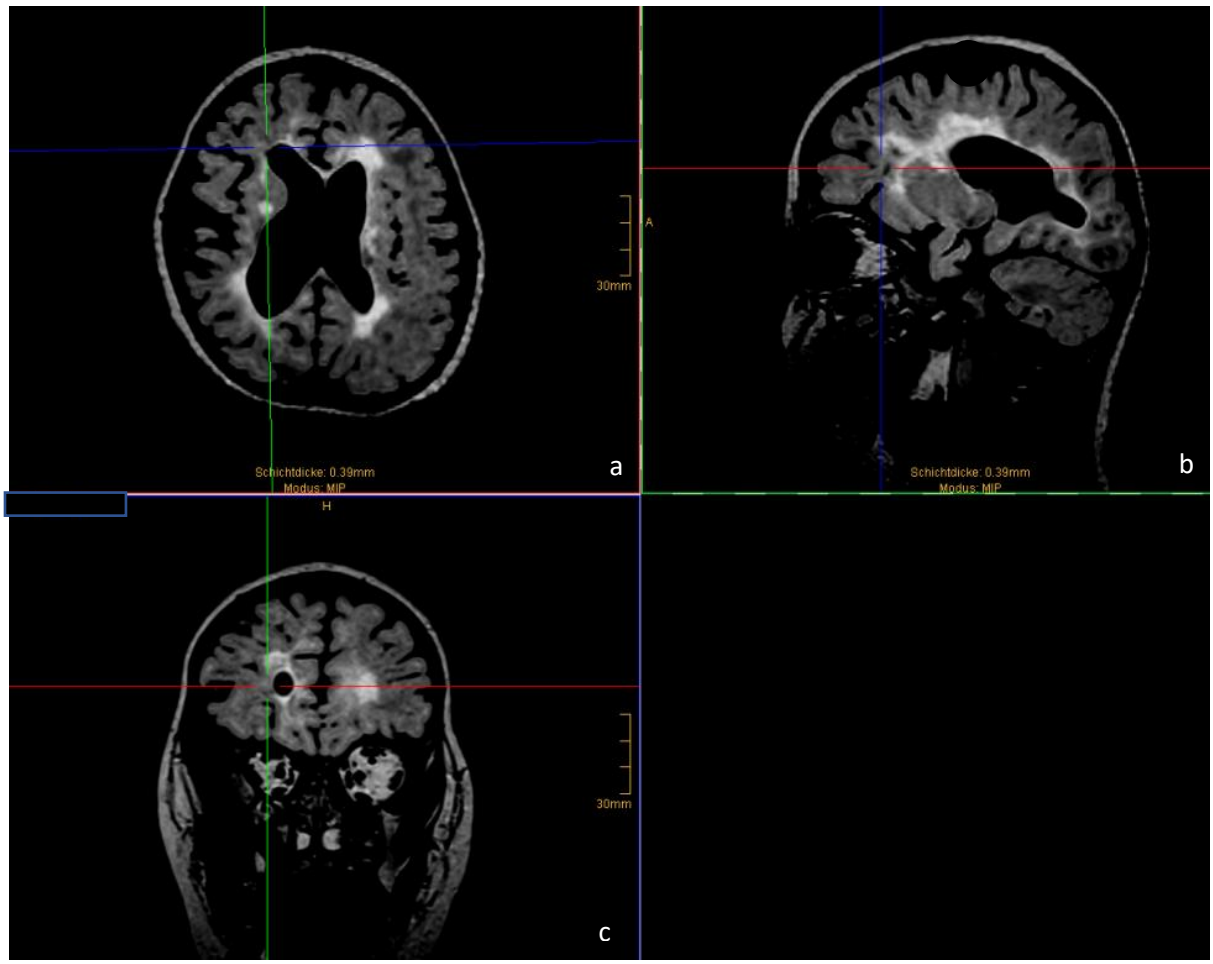
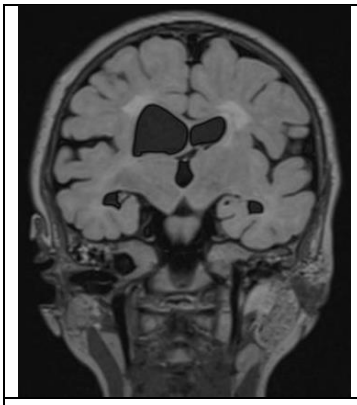
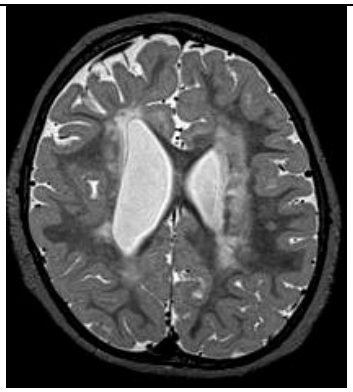


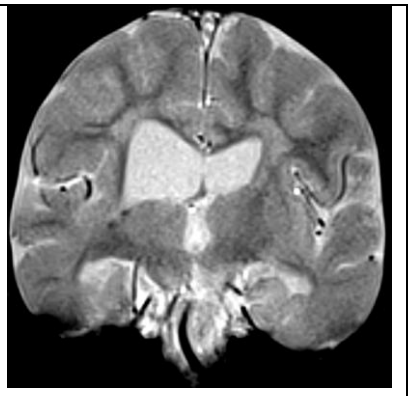
Figure 14. MRI showing reduced corticomedullar differentiation, Pat. 2, at age one year and ten months.
Flair space, coronal sequence, 1mm, a: transversal, b: sagittal and c: coronal section. See pointer in the region anterior to the right frontal ventricle horn (area corresponding to bleeding in Figure 13).



FLAIR, coronal section
Bilat. PVL accentuated on the L, and corpus callosum thinning.



T2, transversal section;
VM on the R, bilateral PVL.



T2 *, coronal section: VM on the R.

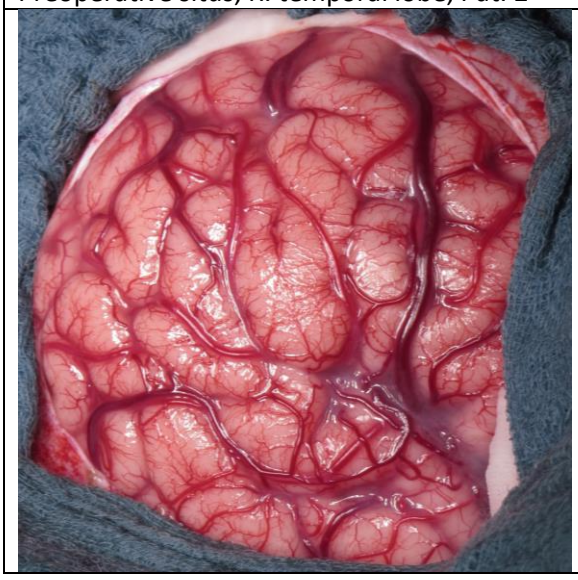
Figure 15. Preoperative MRI, Pat. 2, at age one year and ten months.

3.1.1.2.7 Epilepsy surgery

Palliative epilepsy surgery was performed to stop the encephalopathic course of the epilepsy with electrophysiological and morphological maxima on the right hemisphere. A seizure-free postoperative course was not expected due to the multifocal abnormalities and the severe epilepsy, but a reduction of seizure frequency and a better cognitive development were aimed. It was decided to perform a combination of resective and disconnective procedures in the right posterior hemisphere.

The 2 5/12 years old child was layed supine with fixed head rotated to the left in the Mayfield fixation device. Neuronavigation registration to preoperative MRI data was performed. A trepanation was performed and the dura was opened. See Figure *16*.
A resection of the right temporal lobe was performed. An unco-amygdalo-hippocampectomy on the right was performed. The anterior hippocampus as well as the lateral temporal lobe were embedded for the histologic analysis. After a dysconnection in the sulcus postcentralis of the parieto-occipital lobe, ECoG was recorded and a resection of the supramarginal gyrus was performed. The dura was closed and the bone flap was reinserted.
The operative course was uneventful and surgery showed no complications.

Preoperative situs, R. temporal lobe, Pat. 2



Situs after surgical intervention, Pat.2

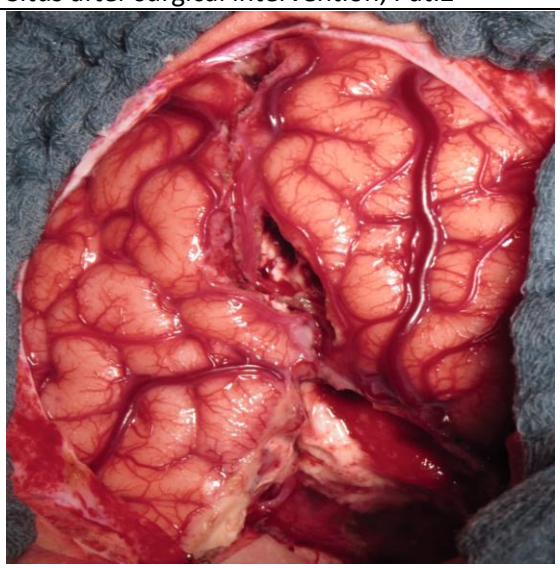
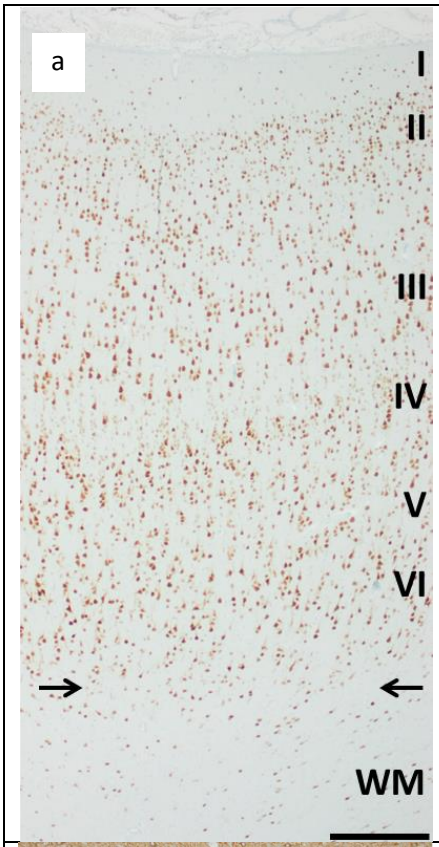


Figure 16. Operative situs, Pat. 2.

3.1.1.2.8 Histology

A mild Malformation of Cortical Development Typ II according to the classification of Palmini 2004 was diagnosed, see also*Table 4.*

Temporal lobe: A subtle increase in gyration was observed, but a polymicrogyria could not be diagnosed. The gray matter-white matter junction is blurred in multiple regions, see Figure *17*.



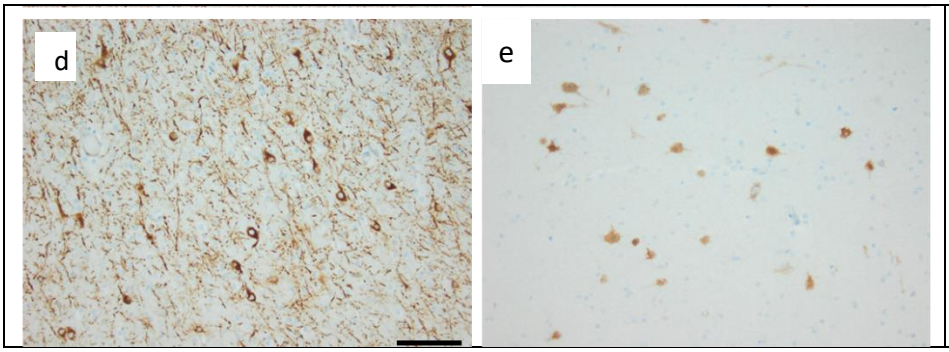
a: NeuN immunohistochemistry shows a regular layering of the cortical ribbon (cortical layers I-VI). Already at low magnification, a blurring of the gray-white matter boundary can be seen (black arrows). An increased number of heterotopic neurons within the subjacent white matter can be observed.

Scale bar in a: 400µm.

b & c: Higher magnification of the gray matter-white matter boundary confirms a blurred gray matter-white matter junction (dotted white line in b) b: MAP-2 immunohistochemistry; c: NeuN immunohistochemistry. Scale bar in b: 200µm, applies also for c.

Figure 17. Histology, Pat. 2: Blurred GM-WM boundary.

A significantly increased number of heterotopic neurons in white matter was found in microscopic and immunohistochemic staining, see Figure *18*.



d & e: Significantly increased density of heterotopic neurons within deep white matter areas.
d: MAP-2 immunohistochemistry; e: NeuN immunohistochemistry.
Scale bar in d: 100µm, applies also for e.

Figure 18. Histology, Pat. 2: Heterotopic neurons.

No dysmorphic cells were found in NFSMI32 staining for intracytoplasmatic neurofilament accumulation. A weak microglia activation was shown with CD68 immuncytostaining. Isolated lymphomonocytic infilitration was detected around blood vessels, but not in parenchyma. GFAP staining showed reactive gliosis in the white matter. Patchy pattern of hypomyelination in white matter was found in standard stain and in MPP staining.
Importantly, no old hemorrhage areas were found in Perl´s Prussian Blue staining.

Hippocampus: No abnormalities, such as neuronal cell loss or gliosis, were observed in standard and immuncytochemic staining.

3.1.1.2.9 Postoperative course

Postoperative course was uneventful.

EEG after surgery
One month after surgery, multiregional epileptic potentials on the left and right hemisphere, continuous slowing of the right hemisphere as well as asymmetry consisting in reduced beta activity and sleep spindles on the right side were detected in EEG.
Twelve months after surgery multiregional spikes of the left and right hemisphere, with maximum in the right frontocentral region as well as a continuous slowing were observed.

Neuroimaging after surgery
MRI showed the subtotal temporal lobectomy and the parietooccipital disconnection, see Figure *19*. Hyperintensities on the resection border of the temporal lobe and on the disconnection border were observed as well as a small bleeding in the occipital horn of the ventricle. A following MRI performed at the age of three years showed no new abnormalities except for slightly increased right ventricle size.

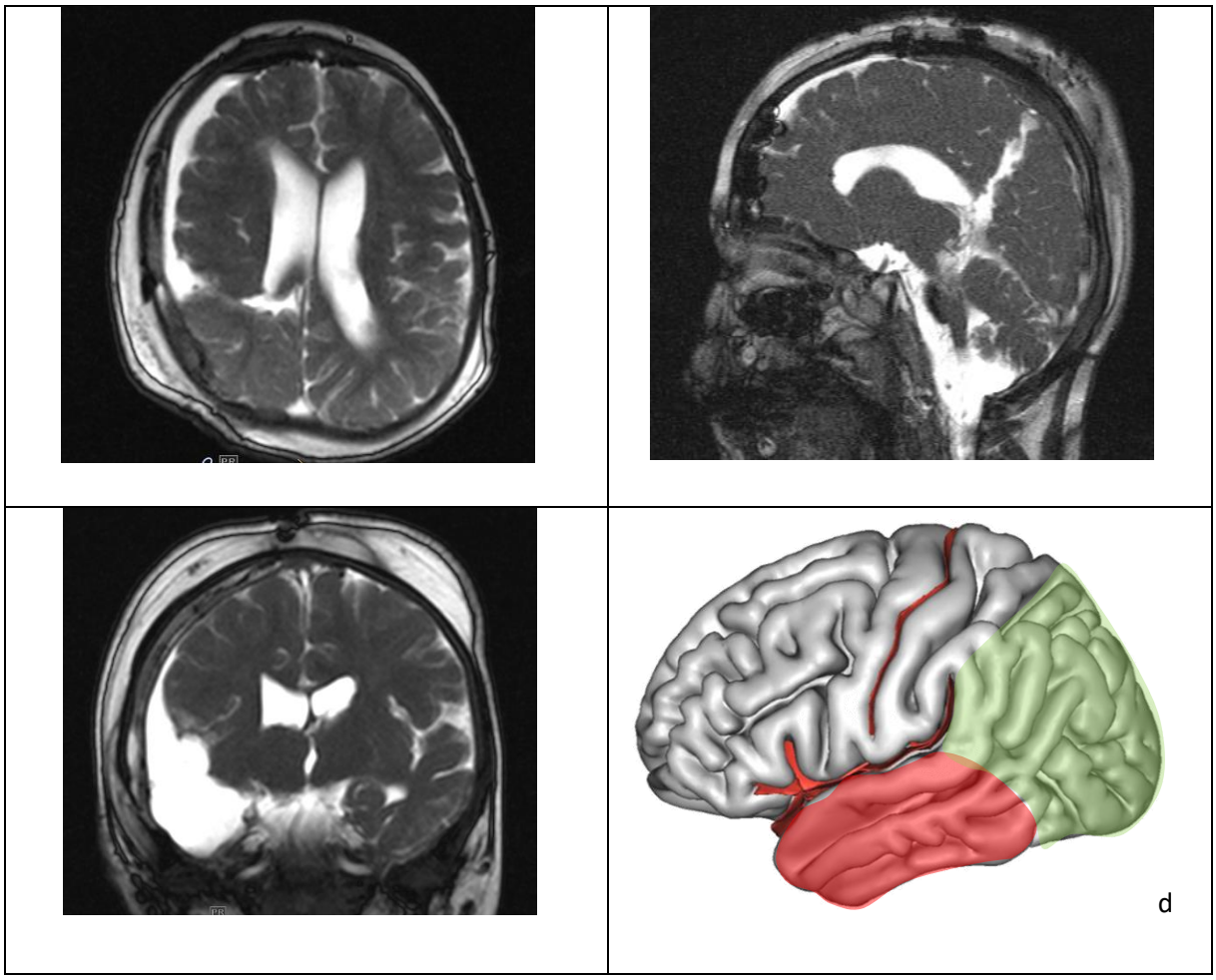


Figure 19. Postoperative MRI, Pat. 2, at age two years and five months.
T2 Sequence, a: transversal, b: sagittal and c: coronal section; d: Schematic reconstruction of resection (red) and disconnection (green).

Epilepsy and outcome after surgery

Three months after surgery the patient showed about 2-3 serial seizures and 2-3 isolated seizures per day. Tonic seizures of the left arm and left leg occurred with tilting of the chest. Sometimes oxygen desaturation to levels of 75-80% was noticed, so that a pulse oxymeter monitoring was started. The medication included oxcarbazepine, clobazam, topiramate and rufinamide.

Twelve months after surgery the patient showed tonic seizures, partially with lateralisation to the left with a frequency of approximatively 10 per day and absence seizures with a frequence of about 5-10 per day. He also showed generalised seizures with deviation of the body to the right and gaze deviation to the right every two weeks. He received sultiam, oxcarbazepine and cannabidiol as regular medication.

Two years after surgery, the patient showed short tonic seizures of the left arm and gaze deviation to the left and the right with a maximum frequency of 5 per day. Outcome was classified as Engel IIIa. The patient was on therapy with vigabatrin, sultiam and cannabidiol. Since the beginning of the therapy with vigabatrin, he had not shown generalised seizures any more. Despite the adverse effects of vigabatrin on visual perception, the therapy was continued because the patient already showed blindness and the therapy had evident positive effects on seizure control.

General development

In the months following surgery the patient was active and in good general condition. He was able to sit independently, to use his left hand as supporting hand and reacted to addressing him with smiling. Proving of perceptive language skills due to impaired cooperation was not possible. Twelve

months after surgery, at about three and a half years age the patient showed muscle hypotonia of the back and bilateral paresis (GMCFS V and MACS III). The patient could use his right hand to grasp and was able to walk with assistance. He was not able to speak but communicated with signs and showed cooperative and friendly behaviour.
Two years after surgery, at about four years age, the patient was not able to speak, but reacted to noises or voices with smiling. He could recognise light stimuli but showed no reaction to other optic stimuli.

3.1.1.3 Patient 3

3.1.1.3.1 Gestation and delivery

No gestational abnormalities were reported. The female patient was born by caesarean delivery on the 38th gestational week because of a pathologic maternal genital smear. She showed a birth weight of 3000 g (34th P), length 56 cm (99th P), head circumference 32 cm (4th P) and APGAR of 9/10.

3.1.1.3.2 Family history and genetics

No neurologic or other diseases were reported in family history. Genetic analysis at age eight years and two months showed a *COL4A2* mutation which was classified as probably pathogenic (probability of 90-99%), c.1856G>A, p-Gly619Asp. This was a *de novo*, heterozygous mutation of a high conserved amino acid in the triple-helix region of the α2 collagen chain. To our knowledge, this mutation has not been previously described in literature.

3.1.1.3.3 Milestones and general presentation

The patient was presented at the Epilepsy Center Vogtareuth at age seven. Developmental delay had been observed beginning at three months age, at a younger age the patient had shown reactive smiling. She learned turning on her back with eight months, sitting at three years age, started crawling at four and she had not learned to walk until present.
At preoperative examinations at the age of eight years and one month, the patient showed microcephaly and right sided hemiparesis (GMFCS IV-V, MACS II-III). She could sit and could crawl or stand with support but could not stand alone. She could use her hands to grasp toys. She could not eat autonomously, would react to music and could not speak or understand spoken conversation. She produced a few syllables. It was not possible to diagnostify a right hemianopia but she used glasses with +10 dpt. She repeatedly showed an aggressive and auto-aggressive behaviour.

3.1.1.3.4 Epilepsy history

The patient developed epilepsy at the age of three months. At the beginning she showed seizures with head and gaze deviation to the right side and loss of consciousness. At eight months age she developed infantile spasms with a maximal frequency of approximately 100 per day. At age of three years she developed generalised tonic-clonic seizures with a frequency of 40 per day. Since then, she showed seizures with gaze deviation to the right side. She showed resistance to following antiepileptic drugs: valproate, topiramate, levetiracetam, vigabatrin, lamotrigin, prednisolone and vitamin B6. The only seizure-free period was observed for four months with valproate.
At eight years age, the patient was on therapy with cannabidiol and showed many myoclonic seizures with a frequency of 10-40 per day, as well as tonic and tonic-clonic seizures without lateralisation of an approximative frequence of 4 per day. Cannabidiol was associated to reduced seizure frequency and stopping generalised seizures but caused sleep disorders and fatigue.

3.1.1.3.5 EEG

EEG showed status of the left hemisphere, with a maximum on the posterior lobe and secondary generalisation to the right side, see Figure *20*. A diffuse continuous slowing with accentuation on the left hemisphere was observed. Ictally, polyspikes in the leftsided hemisphere in the fronto-temporal-parieto-occipital regions and in the rightsided fronto-temporal region were recorded.

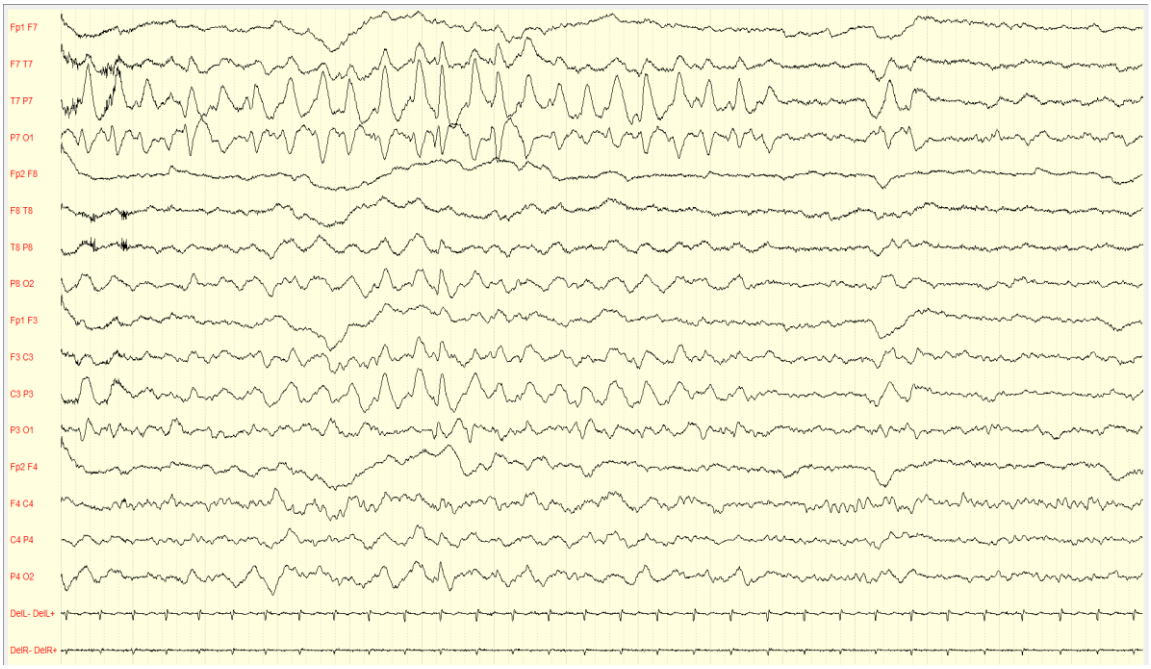


Figure 20. Preoperative EEG, Pat. 3.
Status of the left hemisphere, temporo-occipital region.

3.1.1.3.6 Neuroimaging

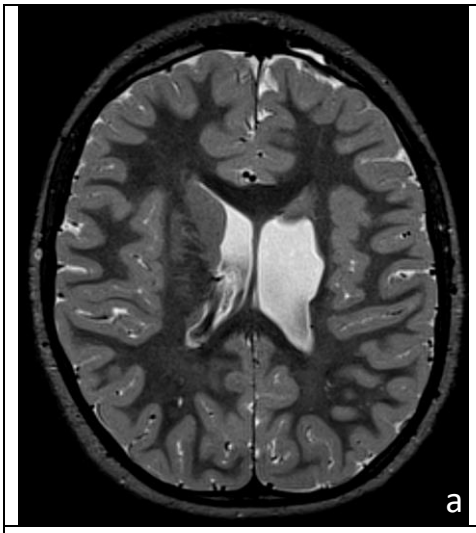
At six months age bilateral periventricular leukomalacia and signs of a putative prepartal hemorrhage or infarction in the left hemisphere were noticed.

At seven years age ventriculomegaly was observed in neuroimaging, with prominent enlargement of the anterior and slight enlargement of the posterior horn of the left ventricle. The left hemisphere showed reduced volume, see Figure *21*. Bilateral periventricular leukomalacia more accentuated on the left side was observed. The terminal vein could not be clearly identified on the left side in contrast angiography. Thus, a venous hemorrage in the subependymal area was suspected.

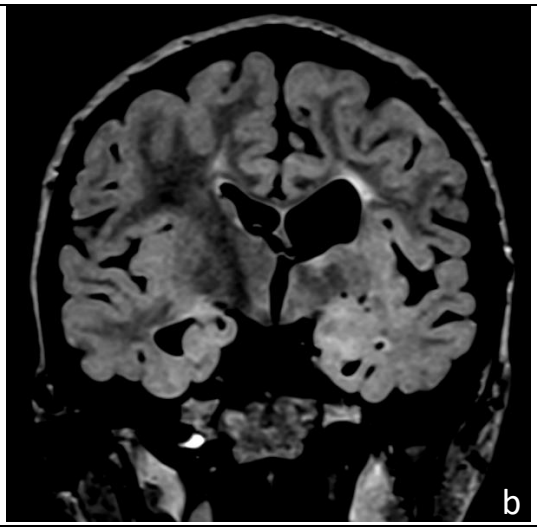
Reduction of volume in left brain stem was observed. Gray matter-white matter differentiation was pathologic, especially in left temporal and occipital lobe and was interpreted as a cortical dysplasia.

Intensity signals in cerebellum, probably corresponding to microbleeds, were detected.

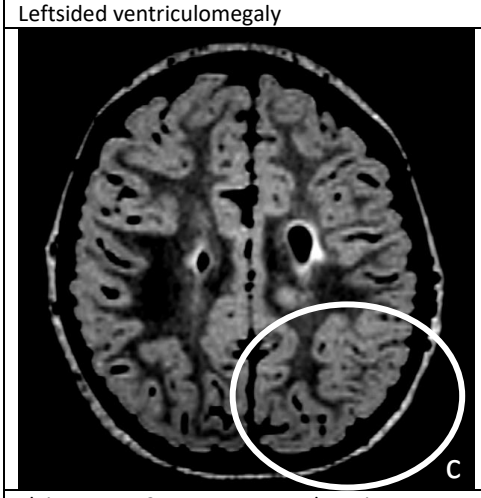
The severe psychomotor retardation of the patient could not be exhaustively explained by the changes in MRI.



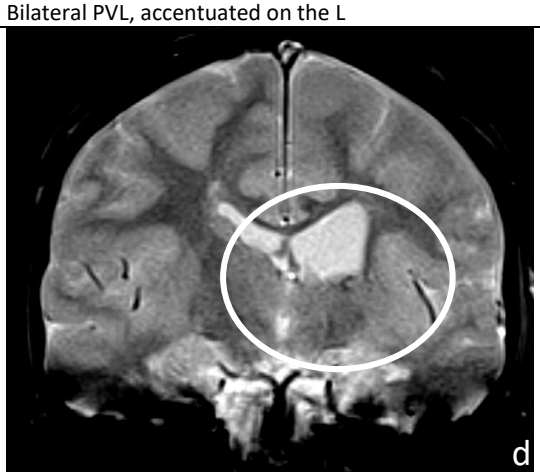
T2 space 0.6 mm, isovoxel, transversal,
Leftsided ventriculomegaly



Flair space 1.2mm isovoxel, coronal,
Bilateral PVL, accentuated on the L



Flair space 1.2 mm, transversal section.
Putatively pathologic corticomedullar differentiation (within the circle), subcortical WM intensity increased in the left hemisphere (LH), volume loss of the LH.



T2 * 4 mm, coronal section. Putatively past hemorrhagic infarction of the L terminal vein/subependymal bleeding in the caudothalamic groove (within the circle).

Figure 21. Preoperative MRI, Pat. 3, at age seven years.

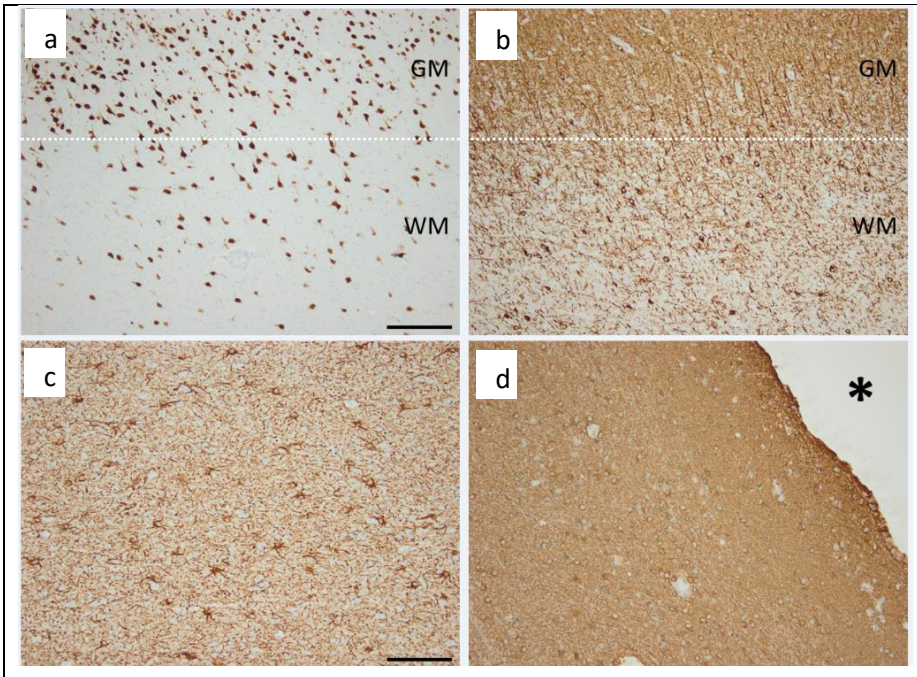
3.1.1.3.7 Epilepsy surgery

Because of EEG and MRI showing epileptogenicity of the left hemisphere, with the perspective of a seizure frequency reduction as well as the possibility of a cognitive development, it was decided to perform a left-sided hemispherotomy.
At 8 9/12 years age, standard hemispherotomy operative procedure with vertical approach was performed.

The patient was layed supine, with her head fixed in a Mayfield device. Neuronavigation registration to preoperative MRI data set was performed. The patient´s skull was trepanated on the middle line of the coronal suture. After opening of the dura, a standard extended vertical parasagittal hemispherotomy (according to the Delalande procedure (Delalande and Dorfmuller 2008)) was performed. The dura was closed and the bone flap was reinserted.

3.1.1.3.8 Histology

Since the operative procedure mainly consisted in a non-resective procedure, only restricted amount of material was available for analysis.
In the resected frontal cortical area, microscopic and immunohistochemic analysis showed six cortical layers. GM-WM junction blurring was found in multiple regions. No dysmorphic neurons or balloon cells were observed. Heterotopic neurons were found in adjacent WM.
In one tissue fragment, GFAP showed severe gliosis in the periventricular white matter. Here, Nissl-LFB staining showed hypomyelination. These findings were interpreted as putatively corresponding to an old venous hemorrhage. No hemosiderin was found. No increased lymphomonocytic infiltrates were found. A blurring of gray matter-white matter junction and periventricular gliosis were diagnosed, which were comparable to a mild malformation of cortical development. See Figure *22*.



a & b: NeuN (a) and MAP-2 (b) immunohistochemistry show blurred gray matter-white matter boundaries with heterotopic neurons within the white matter.
c & d: GFAP immunohistochemistry shows moderate gliosis within the areas depicted in a & b, whereas a prominent periventricular gliosis can be seen in d.

Dotted lines in a & b: gray white-matter boundary. Asterisk in d: ventricle. GM: gray matter. WM: white matter.
Scale bar in a: 200 µm, applies also for b. Scale bar in c: 100 µm, applies also for d.

Figure 22. Histology. Blurred GM-WM boundary and periventricular gliosis, Pat. 3.

3.1.1.3.9 Postoperative course

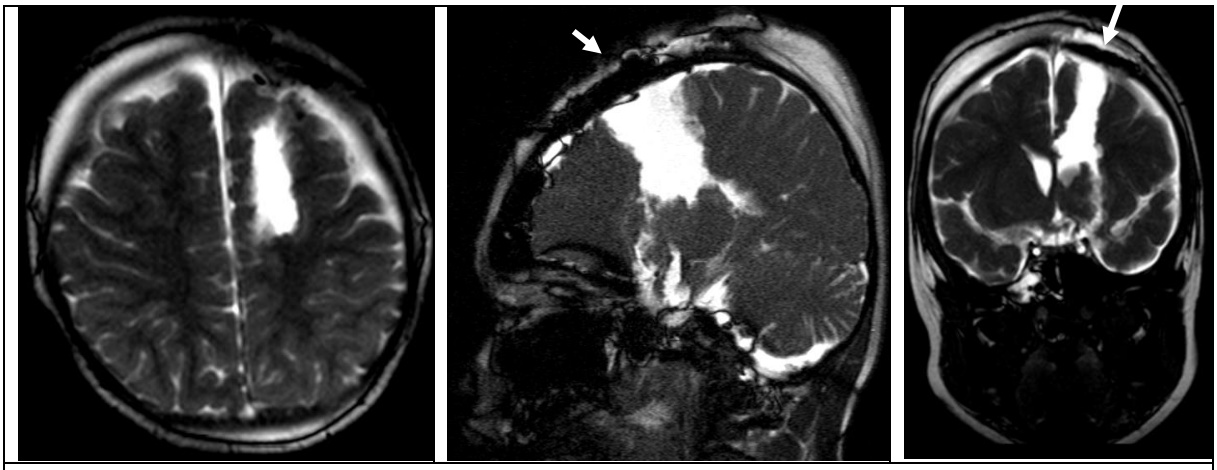
Postoperative course was uneventful. As expected after left-sided hemispherotomy, right-sided hemiparesis and right-sided hemianopia were present.

EEG after surgery

EEG performed six months after surgery showed left-sided and right-sided spikes, a continuous slowing of the left hemisphere and asymmetry consisting of a reduction of β-waves and average activity in the left hemisphere.

Neuroimaging after surgery

MRI after surgery did not show new abnormalities except for small hygromas, see Figure *23*. The hygromas showed consequent regression and a compensatory enlargement of the left ventricle was observed.



Imaging after vertical hemispherotomy and minimal cortical resection, see arrows indicating surgical access.
T2 sequence in a: transversal, b: sagittal and c: coronal section.

Figure 23. Postoperative MRI, Pat. 3 at age eight years and nine months.

Epilepsy and outcome after surgery

The patient showed a single seizure on the fifth postoperative day, since then presenting no further seizures in the following six months. Medication with cannabidiol was stopped and treatment was continued with zonisamide. At follow up examination six months after surgery, stopping of the treatment was planned based on seizure-free outcome. Six months after surgery, Engel-Outcome was classified as Class Ia.

General development

In the months following surgery, the patient showed improved wakefulness, awareness, interaction with her parents, and started speaking syllables. Speech perception could not be assessed due to limited interaction. Aggressive and auto-aggressive behaviour was not shown any more.
The right-sided hemiparesis, expected after hemispherotomy, impaired standing of the patient.
At nine years age, the patient could turn from the supine to the prone position and sit without support. She received speech therapy and physiotherapy and did not attend school but received teaching at home.

3.1.1.4 Synopsis: Histological findings in patients 1-3

In the following table, histologic findings in the patients 1-3 of this cohort are compared.

	Patient 1, I	**Patient 1, II**	**Patient 2**	**Patient 3**
Temporal lobe	Reduced cell density in layer II and III, neuronal cell clusters Microcolumnar organization WM-GM J blurring Heterotopic neurons in WM PV nodular and lentiform heterotopias WM thinning Pseudocyst corresponding to old damage Fibrillar gliosis adjacent to pseudocyst Mild pial fibrotic thickening	Horizontal dyslamination Multifocal WM-GM J blurring Heterotopic neurons in WM Gliosis Perivascular lymphomonocytes No signs of old bleeding/infarction	Subtle increase in gyration WM-GM J blurring Heterotopic neurons in WM Perivascular lymphomonocytes Gliosis in WM No signs of old bleeding/infarction Patchy pattern of hypomyelination in WM	na
Hippo-campus	Segmental neuronal cell loss in PCL New hypoxic-ischemic lesions, probably due to surgery Astrocytic and fibrillar gliosis	na	No abnormalities in available fragments	na
Occipital lobe	Microcolumnar organization Pseudocyst corresponding to old damage Mild pial fibrotic thickening	na	na	na
Frontal lobe	na	na	na	WM-GM J blurring Heterotopic neurons in WM PV gliosis and demyelination in one fragment of tissue, probably due to old damage
Diagnosis	FCD III D Hippocampus Sclerosis NOS	FCD Type III NOS	mMCD Typ II	Blurring of the Gray Matter-White Matter Junction, Periventricular Gliosis

Table 4. Histology findings (Summary).

I: Material from first surgical procedure, II: Material from second surgical procedure. FCD: focal cortical dysplasia, GM: gray matter, J: Junction, mMCD: mild malformation of cortical development, na: not available, NOS: not otherwise specified, PCL: pyramidal cell layer, PV: periventricular, WM: White Matter.

3.1.1.5 Synopsis: Neuroimaging, epilepsy surgery and histology in patients 1-3

In the following *Table 5,* clinical history, relevant findings and surgical procedures in patients 1-3 are summarised.

	Patient 1	Patient 2	Patient 3
***COL4A1/2* gene mutation/Domain/ Pathogenicity/ Inheritance/ Family history**	*COL4A1* c.3985G>A p.Gly1329Ser het. /THX/PD/ Maternal/ Miscarriage and stroke in the mother	*COL4A2* c.4084G>A p.Gly1362Arg het./THX/PD/na *COL4A2* c.4684G>A p.Asp1562Asn het/NC1/VUS/na	*COL4A2* c.1856G>A p.Gly619Asp het./THX/PD/ *de novo/* unremarkable
Gestation /Delivery/Head c.	Hyperemesis gravidarum/Caesarean/ 2.Pc	IUGR, Plagiocephalus IVH II L, I R /Caesarean/<1.Pc	No abnormalities reported/Caesarean/4.Pc
Selected clinical findings	Microcephaly HPa R DEV Strabismus	Microcephaly TPa, accentuated L DEV Strabismus, VPI	Microcephaly HPa R DEV VPI, Hyperopia
Epilepsy syndrome/ Age at onset	Focal epilepsy L H/ 11 mo	Bihemispheric focal epilepsy with max R T-P-O/ 7 mo	Focal epilepsy L H/ 3 mo
Epilepsy semiology	Focal tonic clonic R After I: Absence seizures, drop seizures	Focal tonic clonic L Oxygen desaturation and acrocyanosis Absence seizures Generalised seizures	Bilateral tonic, tonic-clonic, myoclonic seizures, infantile spasms
EEG-monitoring	Interictal: Status L H, max. L T-P Slowing, asymmetry	Interictal: Bilat. Status T-P-O max. R Slowing Ictal: SP T-P-O R	Interictal: Status L H max. T-P-O Slowing, asymmetry Ictal: Polyspikes L F-T-P-O, R F-T
MRI Findings	VM L Bilat. PVL, deep WMC, accentuated R CC thinned Brain stem hypoplasia L Reduction of volume L H Bilateral multiple cortical abnormalities Defect L thalamus Atrophic L hippocampus Putatively microbleeds	VM R Bilat. PVL and deep WMC, accentuated R Leukomalacia L optic radiatio CC thinned Reduction of volume R H Multiple cortical abnormalities R R BG and R thalamus smaller Putatively bihippocampal pathology Disseminated microbleeds in CEB, brainstem, brain parenchyma	VM L Bilat. PVL, accentuated L Brain stem thinned L Cortical abnormalities L Defect caudate ncl. L Putatively past venous hemorrhage L Bilat. hyperintensities in CEB, putatively ischemic changes
Surgery **I: first intervention** **II: second intervention/Age**	I. L T-P-O lobectomy Amygdalohippocampectomy Cingulotomy /40 mo II. L Vertical parasagittal hemispherotomy with minimal cortical resection /13 y	R Unco-Amygdalo-Hippocampectomy Disconnection P-O lobe Resection supramarginal/ 29 mo	L Vertical parasagittal hemispherotomy/8 y
Engel Outcome/ Time after surgery	IIIa/ 9 y after I. intervention IIIa/ 12 mo after II. intervention	IIIa/ 2 y	Ia/ 6 mo
Histology	FCD IIId Hippocampal sclerosis (NOS)	MMCD Type 2	Gray Matter-White Matter Blurring, Periventricular Gliosis

Table 5. Clinical and neuroimaging findings, epilepsy surgery and histology in patients 1-3.

Abbreviations: BG: basal ganglia, Bilat: Bilateral, c: circumference, CC: corpus callosum, CEB: cerebellum, DEV: psychomotor developmental delay, FCD: focal cortical dysplasia, F: frontal, H: Hemisphere, Het: heterozygote, HPa: Hemiparesis, IUGR: intrauterine growth retardation, IVH: intraventricular hemorrhage, L: leftsided, mMCD: mild malformation of cortical development, mo: months, na: not available, ncl: nucleus, NC1: non collagenous domain, NOS: not otherwise specified, O: occipital, P: parietal, Pc: percentile, PD: Probably damaging, PVL: periventricular leukomalacia, R: rightsided, SP: seizure pattern, T: temporal, THX: triple-helical domain, TPa: tetraparesis, VM: ventriculomegaly, VPI: impairment of visual perception, VUS: variant of unknown significance, y: years, WMC: white matter change.

3.1.2 Patients who did not undergo epilepsy surgery

In the following, clinical history, main findings and therapeutic approach in the patients 4-9 are presented, see Table *6*. Selected neuroimaging findings are presented in Table *7*.

3.1.2.1 Synopsis: Clinical history and findings in patients 4-9

Patient	Pat. 4	Pat. 5	Pat. 6	Pat. 7	Pat. 8	Pat. 9
***COL4A1/-2* mutation and domain/ pathogenicity**	*COL4A1* c.625G>A p.Gly209Ser het./THX, VUS-Probably damaging	*COL4A1* c.4981C>T p.Arg1661Cys het./NC1,VUS	*COL4A1* c.2086G>A p.Gly696Cys het./THX, Probably damaging	*COL4A1* c.2591G>A p.Gly864Glu het/THX,VUS	*COL4A1* c.2096G>A p.Gly699Asp het/THX,Very probably damaging	*COL4A1* c.2987G>A p.Gly996Asp, het./THX, Probably damaging
Inheritance/family history	*de novo*/unremarkable	Maternal/ miscarriage of 1.child at 10. GW	*de novo*/ tic disorder in 1. brother, PWS in 2. brother	na/unremarkable	na/unremarkable	*de novo*/unremarkable
Gestation/Delivery/ Head C/Postnatal abnormalities	Prenatally observed VM in US/caesarean/8.Pc/ SGA, Patent ductus arteriosus	No abnormalities reported/vaginal delivery/16.Pc/no abnormalities noticed	Caesarean after abdominal trauma, pathol. CTG/ 4 Pc./ SGA	Vaginal bleedings at the beginning of gestation, Prenatally observed VM in US /vaginal delivery/3 Pc/perinatal cyanosis	Hyperemesis gravidarum/Vaginal delivery with bleeds on 36+1 GW/29 Pc/facial and orbital hematomas, signs of subependymal bleed L, VM R, cystic structures in postnatal US	HELLP Syndrome, IUGR/caesarean/<3 Pc/ CC hypoplasia, cystic defects and VM in postnatal US
Main clinical findings	Microcephaly Muscular hypotonia Persistent foramen ovale DEV	Sec.Microcephaly Mild HP R Muscular hypotonia DEV	Microcephaly Mild HP R DEV	Microcephaly HP R Muscular hypertonia DEV	Sec.Microcephaly Spastic HP L Atactic-dyskinetic HP R DEV	Microcephaly Spastic TP Dysphagia DEV
Ocular findings	Bilat. congenital cataract Bilat. Peters Anomaly	Hyperopia Astigmatismus Strabismus	Bilat. congenital cataract Hyperopia Astigmatismus Strabismus	Partial bilat. optic nerve atrophy Strabismus divergens Pathol. VEP	Bilat. congenital cataract Coloboma R eye	Bilat. congenital cataract
Epilepsy semiology	Atonic s. Head falling with nystagmus Cyanotic s. Infantile spasms	Tonic bilat. s S. with gaze deviation to R/L, nystagmus	Tonic s., myoclonic s. Vomiting and cloni R, then generalisation and Todd paresis R Non-convulsive status Infantile spasms	Tonic s., myoclonic s. Smacking with head and gaze deviation to L Seizures in fencer position	Atonic s. with head and gaze deviation to L, pallor, oxygen desaturation, smacking, vomiting or gaze deviation to R Myoclonic s. Status epilepticus	Myoclonic s. with head deviation to L, bilat. tonic s., vomiting, crying Cyanotic s. with hypotonia
EEG-Monitoring	Interictal: multifocal spikes, continuous status L T-P-O, asymmetry, incomplete hypsarrhythmia, diffuse slowing R Ictal: status termination, fast activity L, beginning of seizures L or not-lateralised	Interictal: EEG-Status L post., R. post. Ictal: Status R post.	Interictal: EEG Status P-O L, slowing P-O L, asymmetry L Ictal: ETPs L P-O	Interictal: multiregional spikes, continuous EEG Status R T-P-O, discontinuous. Status L CT, slowing, asymmetry Ictal: ETP L CP	Interictal: multiregional EEG Status, continuous slowing R, asymmetry	Interictal: EEG Status R T-P-O, ETPs L continuous generalised slowing, asymmetry Ictal: bilat. EEG activity, max. R

Epilepsy syndrome/age at onset	Symptomatic focal bihemispheric epilepsy/ 3 mo	Symptomatic focal bihemispheric epilepsy with biposterior maximum/ 8 mo	Focal epilepsy L PO/ 6 mo	Focal epilepsy L CT and R TPO/ 5 mo	Multifocal bihemispheric epilepsy/ 5 mo	Symptomatic epilepsy R H/ 3 mo
MRI findings	VM L L WM hypoplasia accentuated F Bilat. fronto-opercular PMG Bilat. cortical heterotopias PV heterotopia L SCHIZ component L Defect L caudate nucleus Putatively microbleeds and hemosiderin in CEB Putatively disseminated calcifications	Bilat. VM Bilat. PVL Bilat. deep WMC Volume loss L H Cortical dysplasia, putatively FCD I max. L T-P-O and R O Hyperintensities in BG Hippocampus bilaterally "malrotated"	VM L Bilat. PVL Subcortical leukomalacia R F Volume loss L H, BG and mesencephalon L, signs of Wallerian degeneration L Thinning of CC Cortical dysplasia, putatively FCD I max. L T-P-O Lesion L thalamus and caudate nucleus	Bilat. VM Bilat. PVL Gliosis R TO CC hypoplasia SCHIZ and PMG L	VM R Cystic lesion L ventricle (pore*) Bilat. PVL, acc. R Deep WMC R Loss of volume RH, signs of Wallerian degeneration R Thinning of CC Cortical abnormalities in the whole R H, in the L H only O BG lesions R, Putamen and globus pallidus missing R Lesion L thalamus Hippocampus sclerosis R	Bilat.VM Cystic enlargement L ventricle CC hypoplasia WMC Cortical abnormality R T-P-O Reduced gyration R T-P, Pachygyria R TP observed at age 4 mo Subependymal heterotopias
Arguments against epilepsy surgery	No congruence of findings in EEG (F maxima) and MRI (T-P maxima)	No congruence of findings in EEG (Status L and R) and MRI (max. L) Bihemispheric pathology Pathogenicity of both genetic mutations	Sufficient seizure control with AED	Bilateral epileptogenic areas, adjacent eloquent regions	Multifocal pathology, sufficient seizure control with AED	Very low chances of seizure freedom after surgery
Treatment	Levetiracetam+ Oxcarbazepine+ Topiramate + Vigabatrin, Ketogenic diet	Vigabatrin+ Phenobarbital+VPA	Lacosamide+VPA +Levetiracetam	VPA+Pregabaline+ Clobazam, Vagus-Nerve-Stimulation	Phenobarbital+ Topiramate+Levetiracetam	Oxcarbazepine+Clobazam+ Tetrazepam
Follow- Up/time after presurgical evaluation	Seizures unchanged, ketogenic diet ineffective/6 mo	Additional generalised tonic-seizures associated to fever/4 mo	Not available	Not available	Seizure frequency increased/2 y	Scoliosis, osteoporosis, severe disability, drug-refractory epilepsy/10 y

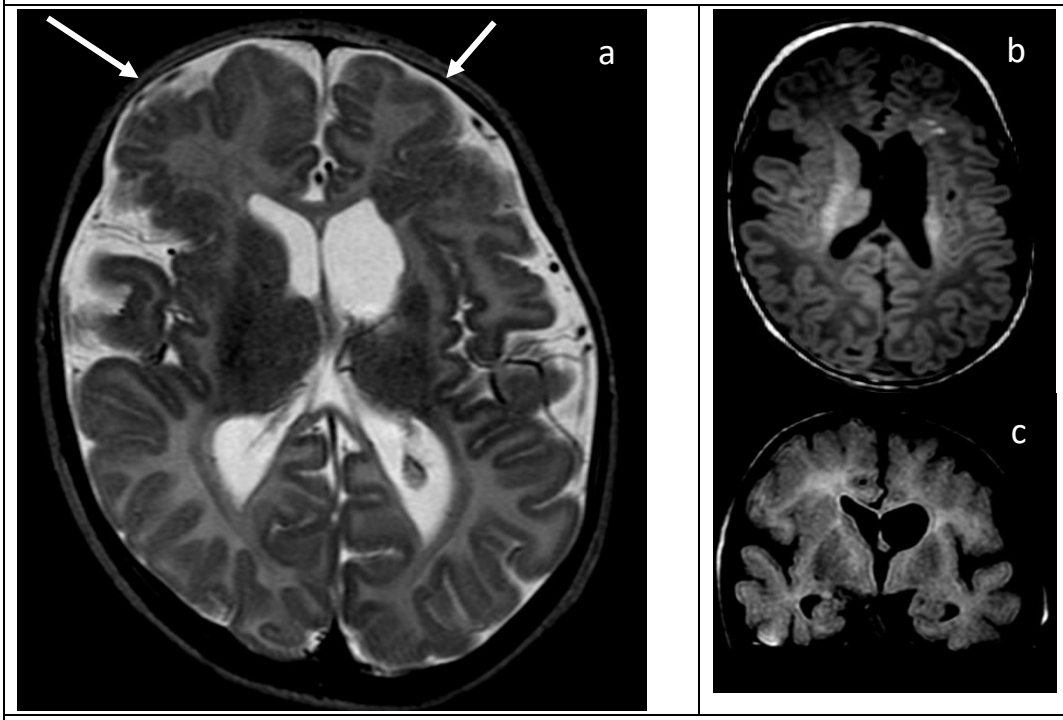
Table 6. Clinical history and findings in patients 4-9.

Treatment: The treatment after presurgical evaluation is reported. Family history is provided if positive inheritance is shown or not-tested. Abbreviations: AED: Antiepileptic drugs, BG: basal ganglia, bilat: bilateral, C: circumference, CC: corpus callosum, CEB: cerebellum, CP: centroparietal, CT: centrotemporal, CTG: cardiotocography, DEV: psychomotor developmental delay, ETPs: epilepsy-typical potentials, F: frontal, GW: gestational week, H: hemisphere, HELLP: hemolysis, elevated liver enzymes, low platelets, IUGR: intrauterine growth retardation, L: leftsided, MCD: malformations of cortical development, mo: months, NC1: non-collagenous domain, O: occipital, P: Parietal, Pc: percentile,*Pore: "Porencephaly", here defined as a local cystic enlargement in ventricle, PMG: polymicrogyria, PVL: periventricular leukomalacia, PWS: Prader-Willi-Syndrom, R: rightsided, s: seizures, SCHIZ: schizencephaly, SGA: small for gestational age, T: temporal, THX: triple helical collagenous domain, US: ultrasound, VEP: visually evoked potentials, VM: ventriculomegaly, VPA: valproate, VUS: Variant of unknown significance, y: year.

Note: additional genetic finding in pat 5: Supernumerary ring chromosome 19 mosaicism, with a possible pathogenicity, and 7: SETBP1 c.4010G>A p. Ser.1337As, probably not pathogenic.

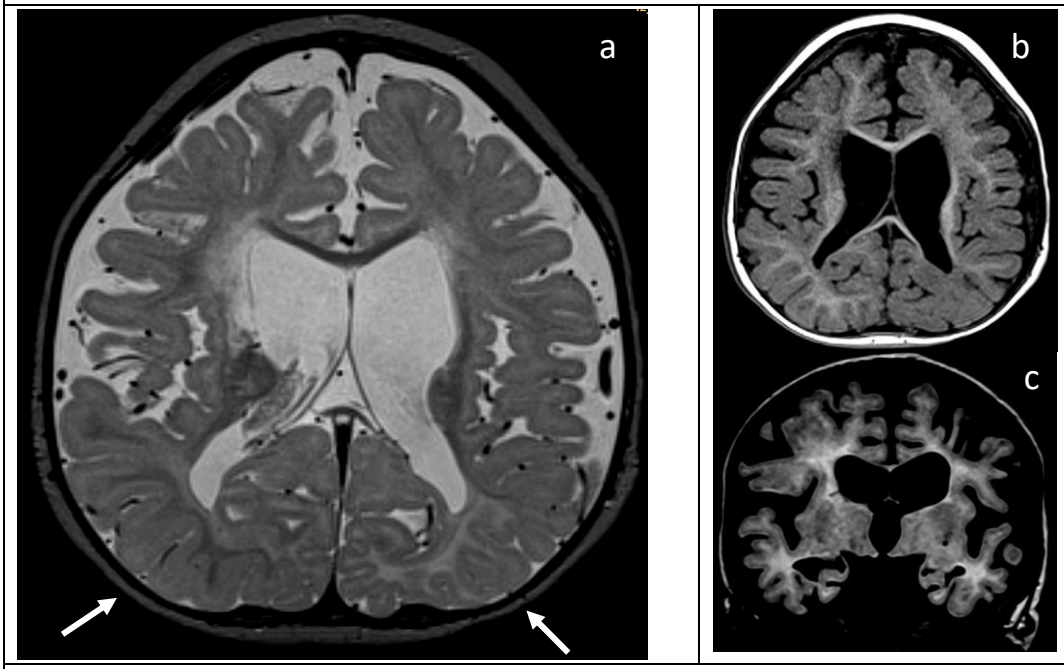
3.1.2.2 Neuroimaging: selected findings in patients 4-9

Patient 4, at age three months



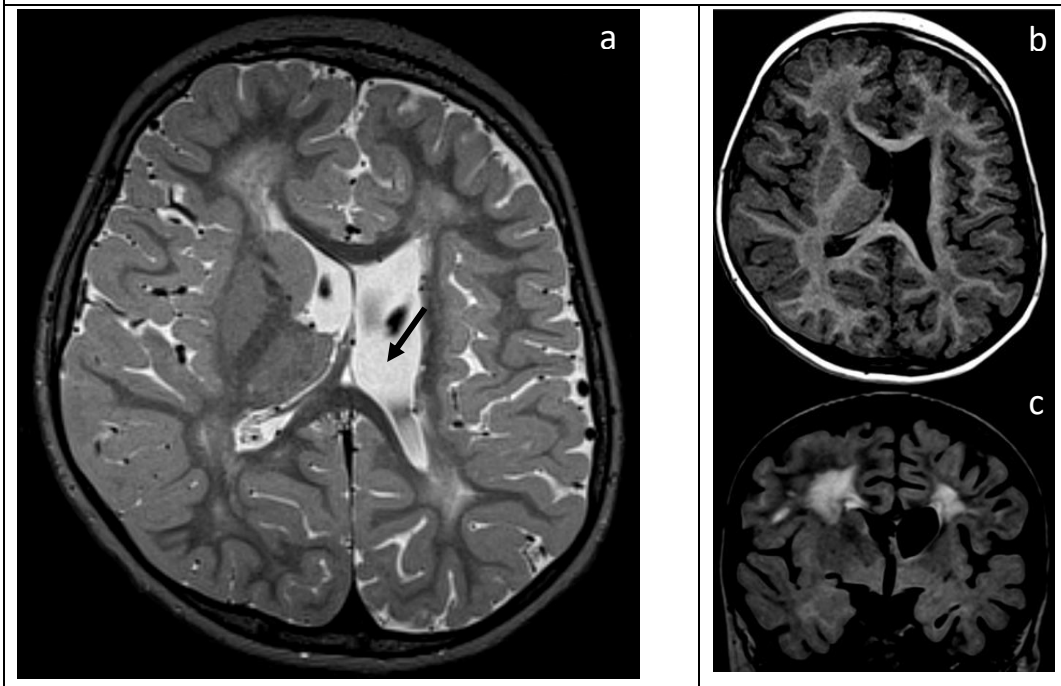
Bifrontal polymicrogyria (see arrows) **cortical and periventricular heterotopia, schizencephaly L frontal**, a: T2 sequence, transversal 3 mm. b: T1 mpr axial section, 1mm: periventricular white matter change. c: FLAIR, coronal, 4mm: L ventriculomegaly, white matter hypoplasia

Patient 5, at age one year and five months



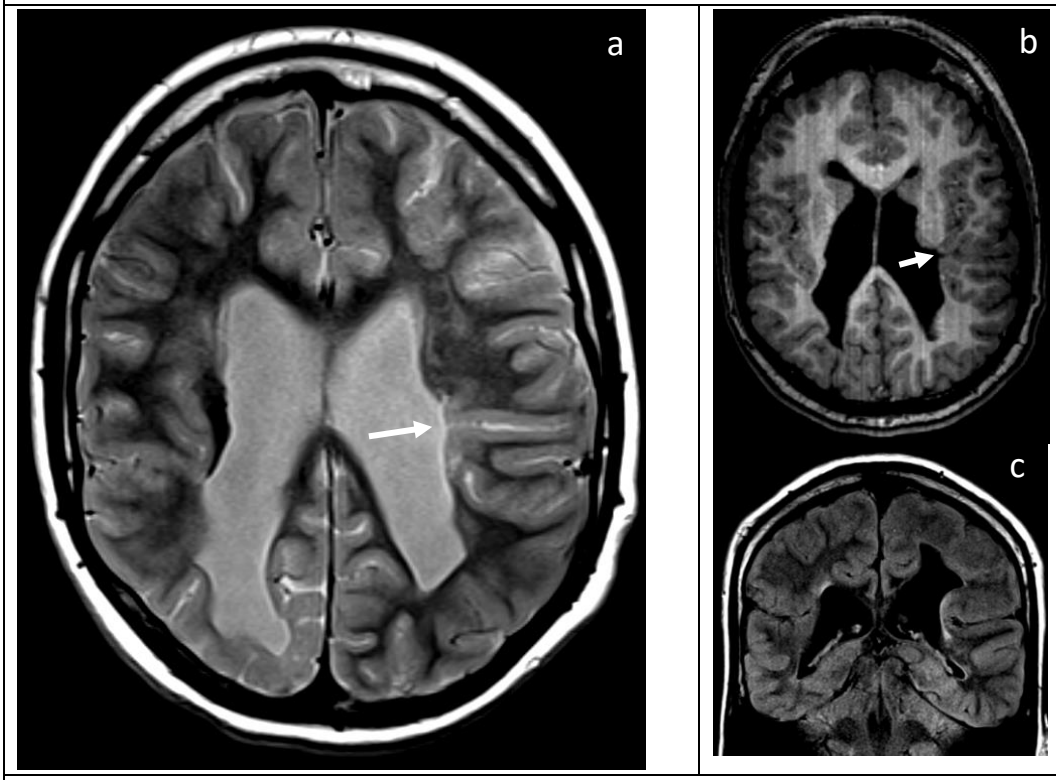
Bilateral ventriculomegaly, abnormal myelinisation/corticomedullar differentiation L occipital, suspected Focal Cortical Dysplasia I. Accelerated myelination R occipital, putatively also here FCD I. (see arrows and difference between L and R hemisphere), a: T2 space 0.6 mm, transversal. b: T1 mpr transversal, 1 mm: periventricular leukomalacia. c: FLAIR space, coronal, 1.2 mm: volume loss in the left hemisphere

Patient 6, at age three years and ten months



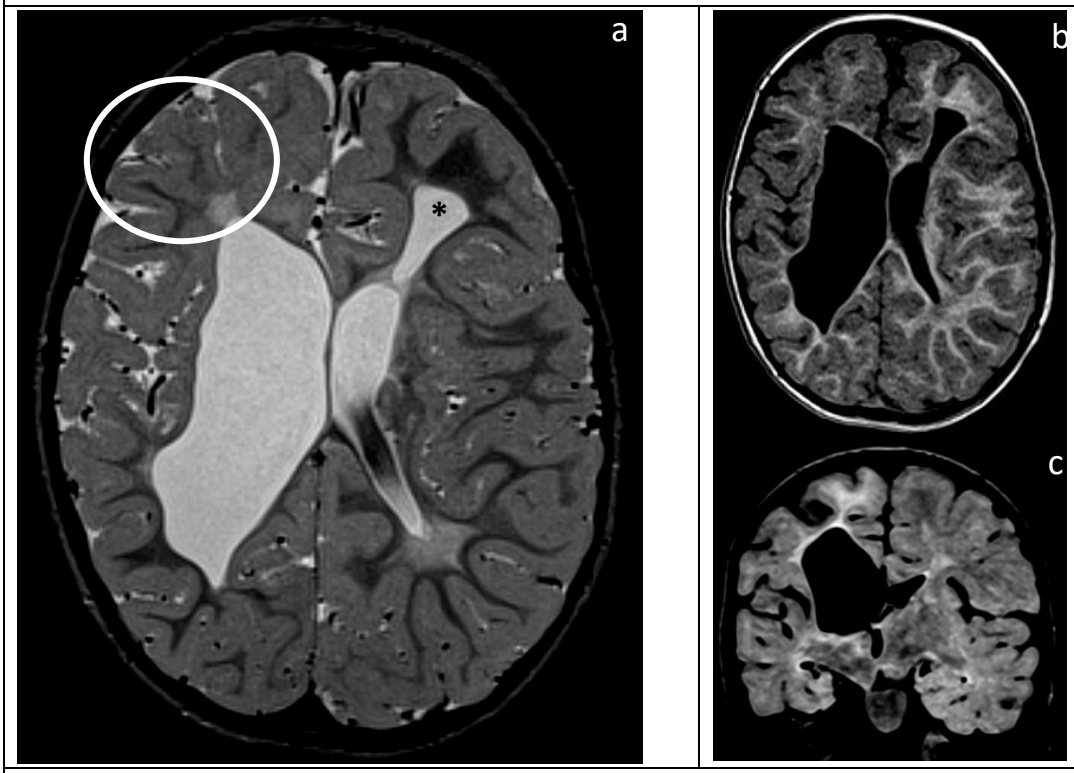
Leftsided ventriculomegaly, L thalamus missing in comparison to R thalamus, see arrow. Putatively FCD I L TPO,
a: T2, transversal, 0.6 mm. b: T1, mpr, transversal, 1 mm: volume loss in the left hemisphere. c: FLAIR space, coronal, 1.2mm: subcortical leukomalacia R F, bilat. periventricular leukomalacia

Patient 7, at age one year and eight months



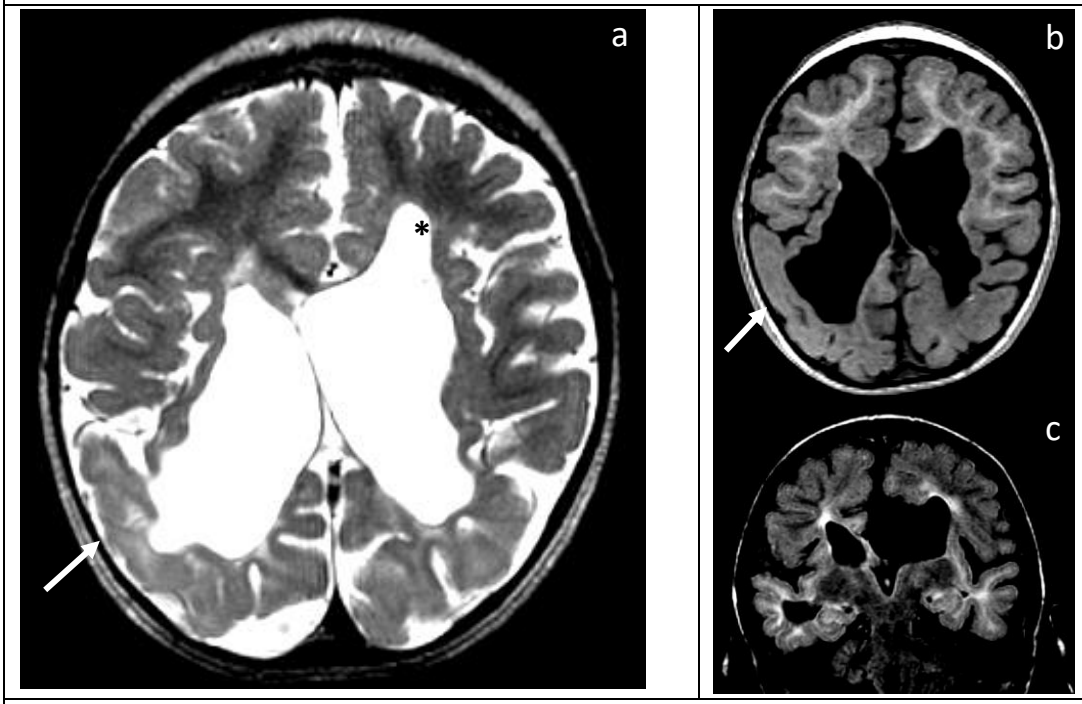
Leftsided schizencephaly and polymicrogyria, see arrows in a and b.
a: T2, transversal, 4 mm: occipital enlargement of ventricle R. b: T1 , transversal, mpr 1mm. c: FLAIR, coronal, 4 mm: corpus callous hypoplasia

Patient 8, at age two years and six months



Ventriculomegaly R, cystic defect anterior to L ventricle ("porencephalic defect"), see asterisk. Pathologic corticomedullar differentiation multifocal in the RH (see area within circle) and L occipital.
a: T2, transversal, 0.6 mm: missing of putamen, globus pallidus R. b: T1, transversal, 1 mm. c: FLAIR, coronal, 1.2 mm

Patient 9, at age two years



Bilat.ventriculomegaly. Pachygyria R T, Cortical dysplasias R TPO, see arrows in a, b. Subependymal heterotopias-
a: T2, transversal 3 mm: cystic defect (see asterisk) communicating with L. anterior ventricle horn, colpocephaly (enlargement of the occipital horns). b: T1 mpr transversal 1 mm. c: FLAIR, coronal 5 mm, see hypoplasia of corpus callosum L

Table 7. Selected neuroimaging findings in patients 4-9.

3.1.3 Overview on phenotype of patients 1-9 with focus on neuroimaging

All presented patients of this cohort showed drug-resistant epilepsy with onset in the first year of life, hemi- or tetraparesis and severe psychomotor developmental delay, as well as ocular impairment. Microcephaly was found in all patients, in two of them developing after birth (patient 5 and patient 8). Renal disease was not observed in our patients. Abnormal blood findings were not observed, except for sporadically raised creatine kinase in pat 1,2,6,7,8,9. For an overview on neuroimaging findings, see Table *8*.

Patient	Ventriculo-megaly	Bilat.PVL	Cortical abn.	Micro-bleeds	Hippoc. pathology	CEB bleeding residuals	BG/Thalamus defects	Prenatal brain injury
1	L	+	Bilat L>R, reduced gyration, pathologic architecture	+	+	+	+	Suspected prenatal L MCA/venous infarction
2	R	+	Multifocal, bilat R>L	+	+	+	+	Bilat IVH
3	L	+	Dysplastic changes L	n.o.	n.o.	+	n.o.	Suspected subependymal bleeding
4	L	+	Bilat PMG, Schiz. L, Bilat heterotopias	+	n.o.	+	+	Prenatal VM in US
5	Bilat	+	Bilat L>R, Putatively FCD Type 1	n.o.	+	n.o.	+	n.o.
6	L	+	Putatively FCD Type 1 L	n.o.	n.o.	n.o.	+	Suspected MCA stroke/germinal hemorrage L
7	Bilat	+	Schiz. L, PMG L	n.o.	n.o.	n.o.	n.o.	Prenatal VM in US
8	R, pore* L	+	Pathologic corticomedullar differentiation bilat R>L	n.o.	+	n.o.	+	Suspected subependymal L and later R parietal bleeding
9	Bilat, cystic entlargement L	+	Reduced gyration, pachygyria R	n.o.	n.o.	n.o.	n.o.	VM and cystic defects in postnatal US

Table 8. Overview on neuroimaging findings in patients 1-9.
BG: basal ganglia, Bilat: bilateral, CEB: cerebellum, Cortical abn.: cortical abnormalities resembling MCDs, Hippoc: hippocampal, IVH: intraventricular hemorrhage, L: leftsided, MCA: middle cerebral artery, n.o.: not observed, PMG: polymicrogyria, Pore*: cystic defect resembling porencephaly, PVL: periventricular leukomalacia, R: rightsided, Schiz: schizencephaly, US: ultrasound, VM: ventriculomegaly.

Remarkably, cortical abnormalities were observed in all our patients and were more frequent than microbleeds.

3.2 Results from literature review

3.2.1 Epilepsy surgery in patients with *COL4A1* mutations in literature

In the following Table *9*, clinical history, main findings and surgical procedures in reported patients with *COL4A1* mutations in literature are summarised.

Reference	**(Papandreou et al. 2014)**	**(Hino-Fukuyo et al. 2017)**	**(Zagaglia et al. 2018)**
***COL4A1/2* Mutation/ Pathogenicity/ Inheritance**	*COL4A1* /nr/nr	*COL4A1* c.4738G>A p.Gly1580 Ser/PD/*de novo*	*COL4A1* /nr/nr
Gestation/Delivery	Di-chorionic di-amniotic twin, caesarean delivery on the 36.GW	Uneventful	nr/nr
Selected clinical findings	DEV, bilat. spastic/dystonic cerebral palsy with R accentuation, strabismus	MC, DEV, mild L HPa, gaze deviation	nr
Epilepsy syndrome/ Age at onset	Structural focal epilepsy/ 2 y	West Syndrome with hemihypsarrythmia/ 6 mo	nr/nr
Epilepsy semiology	Vacant spells with vomits, R arm and leg stiffening, R tonic posturing, epileptic spasms	Spasms in cluster, tonic seizures. After I: clonic seizures of the bilateral upper extremities.	nr
EEG-monitoring	Interictal: Slowing, multifocal discharges midline and R frontal region Ictal: Discharge, Slow wave with attenuation, spike activity over midline and R frontal region	Interictal: continuous high-voltage spikes and waves in right posterior quadrants After I. surgical procedure: continuous discharges in the RH, max P-O	nr
Neuroimaging	Bilat. PVL PV WM atrophy, worse on the L CC thinned Signs of previous germinal hemorrhage	Bilat. VM Increased PV enhancing (T1) Decreased intensity right PV wall (T2) FDG-PET and ECD-single photon emission CT suggesting reduced functioning of the RH	nr
Surgery (technique) I: first intervention II: second intervention/ Age at surgery	Corpus callosotomy/ 6 y	I.: Corpus callosotomy/ 20 mo II.: Vertical parasagittal hemispherotomy with minimal cortical resection/ 28 mo	Resection of a L T-O dysplasia/ 21 mo
Outcome/Time after surgery	Seizure-free/ 4 mo Seizures every 6-8 weeks/ 4 y (as later reported in review of (Zagaglia et al. 2018)	Seizures returned, more frequent spasms/ 1 mo after I Seizure free/ 1 y and 6 mo after II	Seizure free /1 y
Histology	Nr	nr	Cerebral blood vessels showing regular thickness

Table 9. Epilepsy surgery in *COL4A1* mutations in literature.
Outcome and findings as reported in the original publications. Abbreviations: bilat: bilateral, CC: corpus callosum, CT: computer tomography, DEV: psychomotor developmental delay, ECD: ethyl cysteinate dimer, FDG-PET: fluorodeoxyglucose positron-emission tomography, GW: gestation week, H: hemisphere, HPa: hemiparesis, L: leftsided, MC: microcephaly, mo: months, nr: not reported, O: occipital, P: parietal, PD: probably damaging, PV: periventricular, PVL: periventricular leukomalacia, R: rightsided, SVD: small-vessel-disease, T: temporal, VM: ventriculomegaly, y: years, WM: white matter.

3.2.2 MCD in association with *COL4A1/-2* mutations

In this patients cohort, malformations of cortical development were found in histology and in neuroimaging in several patients. To better understand relevance of these findings and possible pathophysiology, a literature review on MCDs in *COL4A1/-2* mutations was performed.

3.2.2.1 Cases reported in literature

Lissencephaly and respectively gyral abnormalities in two patients carrying *COL4A1* mutations with Muscle-Eye-Brain disease had been observed, and mice histology showing abnormal cortical organisation was reported (Labelle-Dumais et al. 2011).

Association of *COL4A1* mutation to schizencephaly in 5 out of 10 patients with schizencephaly was then demonstrated, as well as focal cortical dysplasia in one patient´s histology (Yoneda et al. 2013). Since then, several cases of patients with MRI-diagnosed MCDs were reported, see Table *10*.

In a recent study, authors screened 9 children with MRI-detected polymicrogyria/schizencephaly and found pathogenic *COL4A2* or *-1* mutations in three cases and *COL4A1* mutations of unknown significance in three cases. Five out of six patients with a mutation showed epilepsy, three of them refractory epilepsy (Cavallin et al. 2018).

COL4A1 was recognised as a causative gene for MCD (Barkovich et al. 2012), particularly for schizencephaly (Volpe 2018, Ch. 6).

Study / number of patients	Mutation (domain, pathogenicity) /Inheritance	MCD in MRI	Histo-logy	Other neuroimaging findings	Epilepsy / Onset	Neurology and development
(Labelle-Dumais et al. 2011)/2	*COL4A1* A3046G p.Met1016Val (THX, path)/nr	Cobblestone lissencephaly	nr	Hydrocephalus, Dandy-Walker Malformation, WMC, CEB hypoplasia	nr	WWS
	COL4A1 C3946G p.Gln1316Glu (THX, path)/ paternal, asympt. father	Mild gyral abnormalities	nr	Hydrocephalus, WMC	seizures/nr	unsp. CMD
(Tonduti et al. 2012)/1	*COL4A1* c.1973G>A, p.Gly658Asp (THX)/nr	Unilat. schiz.	nr	WMC, heterotopia, calcifications in BG	nr	TIA at age 23, muscular cramps
(Yoneda et al. 2013)/6	*COL4A1* c.1121-2dupA, p.Gly374_Asn429delinsAsp (Exon 20-22)/paternal, asympt. Father	nr	FCD	Unilat. pore, defects in Thal and BG due to subependymal hemorrhage	+/nr	Hemiplegia
	COL4A1 c.3976G>A, p.Gly1326 Arg (THX)/ *de novo*	Bilat. schiz.	nr	Calcifications, hemosiderin	+/nr	Quadriplegia
	COL4A1 c.3995G>A, p.Gly1332Asp (THX)/ absent in mother	Unilat. schiz.	nr	Calcifications, hemosiderin	+/nr	Hemiplegia
	COL4A1 c.1835G>A, p.Gly612Asp (THX)/nr	Bilat. schiz.	nr	Thin CC, thin brainstem, CEB atrophy, calcification, hemosiderin, multicystic EM	+/nr	Quadriplegia
	COL4A1 c.3245G>A, p.Gly1082Glu (THX)/nr	Unilat. schiz.	nr	Bilat. pore, calcification, CEB hypoplasia	+/nr	Triplegia
	COL4A1 c.3122G>A, p.Gly1041Glu (THX)/nr	Unilat. schiz.	nr	hemosiderin	+/nr	Quadriplegia

(Niwa et al. 2015)/1	*COL4A1* c3976G>A, p.Gly1326Arg (THX)/ *de novo*	Unilat. schiz.	nr	SWI sugg. past hemorrhage, vein tortuosity CT: calcifications	+ /15 mo	nr
(Matsumoto et al. 2015)/1	*COL4A1* mutation not specified/*de novo*	Bilat. schiz.	nr	Cortical atrophy, absence of CC, VM	nr	nr
(Meuwissen et al. 2015)/2	*COL4A1* c.1964G>A, p.Gly655Glu (THX, VUS-path)/inh	Unilat. schiz.	nr	Pore, IVH, CEB hemorrhage	+/nr	Hemiparesis, DEV
	COL4A2 c.3368A>G, p.Glu1123Gly (THX, path)/inh	Unilat. schiz. Overlying CD	nr	nr	nr	DEV, ADHD
(Smigiel et al. 2016)/1	*COL4A1* c.2123G>T, p.Gly708Val (THX, path)/ *de novo*	Bilat. schiz	nr	PV calcifications, agenesis CC, VM	+/4 mo	IUGR, postnatal growth retardation, microcephaly, DEV
(Khalid et al. 2018)/1	*COL4A1* mutation not specified/*de novo*	Unilat. schiz.	nr	Pore	nr	Hemiparesis, microcephaly
(Cavallin et al. 2018)/6	*COL4A2* c.4129G>A, p. Gly1377Arg (THX,path)/maternal, asympt. mother with PV hyperintensity in MRI, previous child with IVH	Bilat. schiz.	nr	Unil. minor VM/pore, bilat. PMG	refractory epilepsy (infantile spasms)/ 5 mo	Spastic tetraplegia, DEV
	COL4A2 c.1776 +1G>A (Exon 24 skipping, path)/ maternal, asympt. mother	Bilat. schiz.	nr	Bilat. VM/pore, heterotopia, pachygyria, bilat. PMG	Focal epilepsy/nr	Spastic tetraplegia
	COL4A1 c.3715 G>A, p.Gly1239Arg (THX, path)/de novo	Bilat. schiz.	nr	Unil VM/pore	Refractory epilepsy (myoclonic and multifocal seizures)/5mo	Spastic tetraplegia
	COL4A1 c.4727C>T, p.Ser1576Leu, (NC1,VUS)/paternal, asympt. Father	Bilat. schiz.	nr	Bilat. VM/pore, mild WMC	Focal epilepsy/60 mo	Hemiplegia
	COL4A1 c.1588C>T, p.Pro530Ser (THX,VUS)/ maternal,asympt mother	Bilat. schiz.	nr	VD, dysmorphic CC	No epilepsy	Spastic diplegia
	COL4A1 c.1085-37T>A (VUS)/paternal, asympt. Father	Unilat. schiz.	nr	Unil. VM/pore, unil PMG	Focal refractory epilepsy /3 mo	Spastic tetraplegia
(Zagaglia et al. 2018)/11*	Mutations not specified	Including: schiz, PMG, FCD, nodular heterotopia	(**)	Including: WMC (putatively vascular insult)	All patients showing epilepsy/nr	Not specified

Table 10. MCDs in patients with *COL4A1/-2* mutations in literature.
Findings are reported with the terminology used in the original publications. For description of the histology, see text. Abbreviations: ADHD: attention deficit hyperactivity disorder, asympt: asymptomatic, BG: basal ganglia, bilat.: bilateral, CC: corpus callosum, CD: cortical dysplasia, CEB: cerebellar, CMD: congenital muscular dystrophy, unsp: unspecified, CT: computed tomography, DEV: developmental delay, EM: encephalomalacia, FCD: focal cortical dysplasia, inh: inheritance, IVH: intraventricular hemorrhage, mo: months, MCD: malformation of cortical development, nr: not reported, path: pathogenic, pore: porencephaly, PMG: polymicrogyria, PV: periventricular, Schiz: schizencephaly, sugg: suggesting, SWI: susceptibility weighted imaging, Thal: thalamus, THX: triple helix, TIA: transient ischemic attack, unil.: unilateral, VD: ventricular dilatation, VM: ventriculomegaly, VM/pore: finding described with both terms in article, VUS: variant of unknown significance, WMC: white matter change, WWS: Walter-Warburg-Syndrome, Epilepsy and onset: +: epilepsy, seizure type with age of onset, if reported. *: including one patient reported in this study (Patient 4), **: histological analysis of cerebral blood vessels in one patient provided, see Table 9.

Table *10* shows in total 32 patients with a *COL4A1/-2* mutation (in three cases a *COL4A2* mutation, in eighteen cases with a *COL4A1* mutation, in eleven cases with a not specified *COL4A1* or *-2* mutation) and neuroimaging finding of a MCD. Most commonly, bilateral (9/32 patients) or unilateral (9/32 patients) schizencephaly was observed. In further cases, schizencephaly was observed, without further specification. In four cases, polymicrogyria was observed, and in one case, cobblestone lissencephaly was detected. Moreover, pachygyria was detected in one case. In 26 out of 32 cases, seizures or epilepsy were reported in association with *COL4A1/-2* mutations and MCD.
These findings support the hypothesis that *COL4A1/-2* mutations can lead to abnormalities of cortical development, which are mostly associated with epilepsy.

3.2.3 Pre/perinatal brain injury in association with *COL4A1/-2* mutations

In several patients of our cohort, intracerebral hemorrhage was present even in case of caesarean delivery. Therefore and depending on neuroimaging findings, prenatal brain injury was suspected in several patients of our cohort.

Variability in finding schizencephaly, MCDs or porencephaly in patients with *COL4A1/-2* mutations, may be due to brain injury in different developmental stages. To support this hypothesis, reports in which timing of prenatal injuries in *COL4A1/-2* mutations is demonstrated were analysed, see Table *11*.

3.2.3.1 Cases reported in literature

Prenatal onset of parenchymal hemorrhage in patients was first reported in two siblings with *COL4A1* mutation, based on cranial ultrasound and MRI-findings on first postnatal day (Vries et al. 2009). Later, several case reports on patients showing *COL4A1* mutations (Table *11*) demonstrated prenatal ICH or stroke through intrauterine imaging.

In two carriers of *COL4A1* mutations, post-mortem histology of neonates was available. In the first case reported, cerebral hemorrhagic lesions and neuroglial heterotopia with vascular hyperplasia, as well as cerebellar peduncles hypoplasia and heterotopia of cerebellar Purkinje cells was found. In the second case, multiple foci of necrotic tissue were found (Colin et al. 2014).

In two further patients with *COL4A1* mutations, pathological post-mortem examination showed severe ventriculomegaly and tissue atrophy, absence of rostral part of corpus callosum, atrophy of basal ganglia, supra- and infratentorial vessel tortuosity (Meuwissen 2011) and intracerebral hemorrhage grade IV, hemorrhages in liver, thymus and adrenal glands (Garel et al. 2013) respectively.

Study / number of patients	*COL4A1* mutation / Inheritance	Gestational week / Ultrasound or cMRI /cCT	Epilepsy and neurology	Other findings
(Meuwissen 2011) / 3	c.2545G>T p.Gly808Val/ *de novo*	27 GW, US: asymmetric VM and extensive cerebral infarction Postnatal US and postmortem MRI: extensive VM and tissue atrophy, poorly gyrated cortex, BG, Thal and CEB atrophy, putatively after venous infarctions.	Seizures after birth	Apnea, poor feeding. Death at 10 d PME
	c.2716 + 1G>A */de novo*	37 GW: VM and severe CEB atrophy Postnatal SWI: low signal intensity on the ventricular margins suggestive of antenatal hemorrhage	Progressive hydrocephalus	Ocular involvement Death at 11 w
	c.3022G>A p.Gly1008Arg /maternal mosaicism in blood cells	26 GW, US: asymmetric VM MRI: massive cerebral infarction Postnatal US, MRI: severe VM and small CEB	Seizures and cerebral palsy	Ocular involvement Apnea and poor feeding at birth
(Vermeulen et al. 2011)/2	c.2245G>A p.Gly749Ser /paternal Father: mild WM abnormalities.	28 GW, US: asymmetrical loss of cerebral tissue, widening of lateral ventricles 29 GW, MRI: VM, focal cerebral mantle defect, IVH Postnatal MRI,1. we: developed hemorrhage in BG	Neonatal seizures, progressive hydrocephalus	Premature birth (31 GW). Death at 10 mo
	c.4150G>A p.Gly1384Ser */de novo*	37 GW, US: decreased head circumference, slightly enlarged ventricles 38 GW, US: VM, IVH, bilat. focal cerebral tissue loss Postnatal MRI, at 1 mo: bilat. focal cerebral tissue loss, affection of BG and CEB	Therapy-resistant epilepsy, DEV	nr
(Lichtenbelt et al. 2012)/1	p.Gly 1103Arg */de novo*	21 GW, US: one-sided germinal hemorrhage with thalamus involvement, PV echogenicity 23 GW, MRI: organising hematoma, destruction of CEB hemisphere Repeat US: VM, cystic evolution of thalamus Neonatal MRI: cavitation BG, absence CEB hemisphere	Hemiplegia	Ocular involvement
(Tonduti et al. 2012)/1	c.2159 G>A p.Gly720Asp/ nr	23 GW, US: IVH Postnatal MRI, 2 m: pore R, WMC L, abnormal BG Postnatal CT, 2 m: bilat. PV calcification	Generalised seizures controlled under ethosuximide, spastic-dystonic TP, sensorineural deafness	Ocular involvement
(Garel et al. 2013)/ 2 siblings	p.Gly188Glu in subsequent pregnancies/ maternal. Mother: arterial retinal tortuosity	25 GW, US: bilat. VM 27 GW, US: bilat. VM, hyperechogenic walls, IV blood clots. 28 GW, US: bilat. VM and hemorrhage, massive one-sided subependymal hemorrhage extending to ventricle, BG and parenchyma 28 GW, MRI: bilat. IVH	nr	Term.o.Preg. PME
		Serial fetal US normal	Normal development at 9 mo	nr
(Takenouchi et al. 2015) / 1	c.3715G>A, p.Gly1239Arg /paternal. Father: HANAC Syndrome.	33 GW, US: cystic lesions postnatal MRI: porencephaly	Seizure at 15 mo, hemiparesis	Arrested fetal growth, jaundice, anemia.

(Colin et al. 2014)/ 2	c.2317G>A p.Gly773Arg /maternal, first daughter with bilat. cataract.	23 GW, US: one-sided hyperechogenic lesions in BG and VM 32 GW, MRI: subependymal hemorrhage 33 GW, US: subependymal hemorrhage, dilated anterior horns of ventricles, CEB hypoplasia	nr	Term.o.Pre. Ocular involvement PMH
	c.3005G>A p.Gly1002Asp /nr	31 GW, US: one-sided hyperechogenic hemisphere and thalamus lesions 32 GW, US: one-sided PV hemorrhage, VM	nr	Term.o.Pre. Ocular involvement PMH
(Matsumoto et al. 2015)/ 1	*COL4A1* mutation not specified/ *de novo*	21 GW, US: unil. VM 25 GW, US: bilat. VM, hyperechogenic lesions 28 GW, US: open clefts, schiz Postnatal cCT: bilateral open schiz, cortical atrophy, absence of CC	nr	Hemolytic anemia
(Harada et al. 2017)/ 1	c.4843G > A p.Glu1615Lys /nr	30 GW, US: VM 32 GW, US: bilateral clefts, calcification, hemosiderosis, pore Postnatal MRI, d 10: pore, cCT: PV calcifications	Symptomatic epilepsy	Ocular involvement; GER, laryngomalacia
(Durrani-Kolarik et al. 2017) / 1	p.Gly785Glu /*de novo*	16 GW, US normal 31 GW, US: fluid-filled frontal lobes 32 GW, US: near complete loss of frontal, temporal and parietal cortex and WM Postnatal MRI: cystic encephalomalacia, CEB encephalomalacia and hemorrhagic debris, suggesting hemorrhagic infarctions	Hydrocephalus with need for shunting, DEV	Ocular involvement
(Khalid et al. 2018)/ 1	Not specified mutation in intron 9/ *de novo*	19 GW, US: one-sided echogenicity 21 GW, MRI: diffusion restriction in same area sugg MCA ischemic infarction. Susceptibility foci and in same area suggesting hemorrhagic transformation of infarction. 35 GW, MRI: focal volume loss, parenchymal cleft extending to ventricle (open-lip schiz). Postnatal MRI at 4 we: volume loss, schiz and pore in affected region.	At 6 mo microcephaly and hemiparesis	No structutal ocular, cardial, renal abnormalities found
(Sato et al. 2018)/1	c.2645_2646delinsAA, p.Gly882Glu/ *de novo*	28 GW, US: bilat. VM 32 GW, MRI: unilat. schiz, bilat. VM Postnatal MRI, day 1: unilat. schiz, multiple bilat. defects suggesting recurrent bleeds in BG and VW; SWI suggesting hemosiderin deposition	Epilepsy from postnatal day 10, rigospasticity	Ocular involvement

Table 11. Prenatal brain injury in patients with *COL4A1* mutations in literature.
Abbreviations: BG: basal ganglia, bilat: bilateral, CC: corpus callosum, cCT: cranial computer tomography, CEB: cerebellum, d: days, DEV: developmental delay, GER: gastro-oesophageal reflux, GW: gestational week, HANAC: Hereditary angiopathy with nephropathy, aneurysms and muscle cramps, IV: intraventricular, IVH: intraventricular hemorrhage, L: leftsided, MCA: middle cerebral artery, mo: months, MRI: magnetic resonance imaging, nr: not reported, PME: post-mortem examination. PMH: post mortem histology, pore: porencephaly, PV: periventricular, R: rightsided, schiz: schizencephaly, sugg: suggesting, SWI: susceptibility weighted imaging, Term.o.Pre.: termination of pregnancy, thal: thalamus, TP: tetraparesis, US: ultrasound, VM: ventriculomegaly, VW: ventricle wall, we: weeks, WM: white matter, WMC: white matter change. For PMH and PME, see text. All reported mutations are heterozygous COL4A1 mutations.

In our literature review (see Table *11*), prenatal brain injury of ischemic or hemorrhagic type was found in total in 17 patients showing a *COL4A1* mutation. In four out of 17 cases the injury was documented with post-mortem examination. In eight out of 17 cases, seizures or epilepsy were reported. No report documenting brain injury during the prenatal period in patients harboring *COL4A2* mutations was found.

The case reports summarised in Table *11* show that *COL4A1* mutations can lead to ischemic and hemorrhagic pre- and perinatal injury, in different brain regions and with variable timing, so that neuroimaging results and clinical outcome may differ. The timing of brain injury in patients with *COL4A1/-2* mutations is discussed in Ch. 4.1.3.

4 Discussion

In this chapter, answers to the study questions as presented in Chapter 1.1 are provided.
First, the phenotype of patients with *COL4A1/-2* mutations according to our clinical findings is explored in Ch. 4.1.1.
In the sections 4.1.2 and 4.1.3, neuroimaging findings, particularly the detection of cortical abnormalities in all our patients, and the timing of the cerebral lesions are highlighted considering results from our cohort as well as our literature review on MCDs and prenatal brain injury in patients with *COL4A1/-2* mutations.
The evaluation of the above mentioned aspects enabled us to identify diagnostic clues which are pointed out in section 4.1.4.
Important issues regarding candidacy for epilepsy surgery and outcome after surgery are analysed in Ch. 4.2 and 4.3. Moreover, histological findings provided valuable hints for the better understanding of pathophysiological aspects and contributed to the elaboration of a model for epileptogenesis in *COL4A1/-2* mutations (Ch. 4.4).

4.1 Which phenotype is observed in *COL4A1/-2* epilepsy patients?

Finding typical patterns in the phenotype of patients carrying *COL4A1/-2* mutations is helpful for diagnosis, clinical management and to better understand *COL4A1/-2* related epilepsy. In this section, clinical findings, genotype-phenotype correlation, neuroimaging and aspects related to timing of CNS injury will be examined. At the end of the section, diagnostic clues are summarised.

4.1.1 Clinical and family history of the patients: what are common findings?

Clinical manifestation of the *COL4A1/-2* mutation in the presented patients included CNS and extra-CNS pathologies, which are considered in the following.

4.1.1.1 CNS Involvement

Epilepsy, severe developmental disorder, motoric dysfunction and microcephaly are important features in the phenotypical spectrum associated with *COL4A1/ -2* mutations in our patients.

Epilepsy: In all patients of our study, epilepsy began in the first year of life, and in six out of nine in the first six months. In our cohort, epilepsy semiology included focal tonic, myoclonic, tonic-clonic and non-classified seizures as well as infantile spasms, seizures with secondary generalization and status epilepticus. Importantly, all patients showed a severe drug-resistant epilepsy. Reports on epilepsy semiology in patients with *COL4A1/-2* mutations included infantile spasms (Shah et al. 2012), focal seizures (Ha et al. 2016), status epilepticus (Leung et al. 2012) and generalised tonic-clonic seizures (Zagaglia et al. 2018).
The observed wide range of epileptic phenotypes in our patient cohort and in literature correlates with the broad morphological range of MRI findings. It is remarkable that in all patients of our cohort, cortical abnormalities could be observed in MRI (for a discussion of neuroimaging findings, see 4.1.2). Our literature review on MCDs in patients with *COL4A1/-2* mutations (see *Table 10*) confirms the relevance of cortical abnormalities in *COL4A1/-2* associated epilepsy.
Clear EEG foci (patients 1, 3, 6) as well as multiregional EEG foci and bilateral pathology (patients 2,5,7,8,9) or epileptic foci in MRI-negative areas (patients 4,5) could be detected in our patients, see *Table 5* and Table *6*. These findings suggest that a diffuse network impairment is underlying, as already proposed in the article of John et al. 2015, see also Ch. 4.4.2.

Development: All our patients showed, depending on their age, severe psychomotor developmental delay or intellectual impairment. Our findings are consistent with previous observations. A first report on developmental delay associated to IUGR, microcephaly and *COL4A1* mutation was published 2016 and similarity to TORCH infection was observed (Smigiel et al. 2016). According to a recent review, intellectual impairment was found in most patients carrying *COL4A1/-2* mutations

with epilepsy (in 39/55 previously published and 36/38 new cases presented by the authors) (Zagaglia et al. 2018).
We propose that developmental delay could be caused by the constant epileptic discharges, which were observed in interictal EEG in all our patients. Moreover, cerebral injury has been reported during the stages of cortical organisation in patients harboring *COL4A1* mutations (see *Table 11* for our review of literature and 4.1.3 for a discussion of the findings). Therefore, we suggest that diffuse impairment of brain development before birth may be an additional factor contributing to developmental delay associated with *COL4A1/-2* mutations, since cortical changes and defects in connecting fiber tracts can lead to severe cognitive disability. In addition, based on the case of patient 3 of our cohort, in whom detected MRI abnormalities were considered not-concordant with the severity of intellectual impairment, we suspect that the impairment of the cortical network in patients with *COL4A1/-2* mutations may be more extended than morphologic changes in MRI.

Motor dysfunction was observed in all our patients. Hemiparesis or tetraparesis, as well as muscular hypo- or hypertonia could be found in our cohort (see Table *5* and Table *6*).
Similar findings were observed in previous studies: motor abnormalities reported in *COL4A1/-2* epilepsy patients included hemiparesis, tetraparesis (Meuwissen et al. 2015), abnormal muscle tone at birth (Smigiel et al. 2016; Zagaglia et al. 2018) spasticity and dystonic features (Zagaglia et al. 2018).
We propose that CNS injury is the main cause of motor dysfunction in patients carrying *COL4A1/-2* mutations. White matter lesions, as well as hypomyelination in the pyramidal tract (as seen in patient 1 of our cohort) can lead to paresis, while basal ganglia damage can lead to atactic-dyskinetic features, as seen in patient 8 of our cohort. Moreover, combination of these lesions and impaired developmental ability make it difficult for patients to develop motoric skills, as observed in patient 3 of our cohort, who could not walk at the age of 8 years.
Nevertheless, a further mechanism associated with motor dysfunction in patients with *COL4A1/-2* mutations could be the direct impairment of skeletal muscles. This mechanism was proposed in a study on a *COL4A1* mutant mice model for the HANAC syndrome, in which abnormal muscle BM and endothelial cell defects of the muscle capillaries were detected (Guiraud et al. 2017). Thus we find it conceivable, that both CNS lesions and muscle defects may be underlying motor dysfunction in patients with *COL4A1/-2* mutations.

Microcephaly: Microcephaly was observed in all our patients and was either already present at birth (in 7 out of 9 patients) or was developed after birth (in 2 out of 9 patients). Microcephaly can result from impairment at the stage of neuronal and glial proliferation or apoptosis (Barkovich et al. 2012). In patients carrying *COL4A2* mutations microcephaly at birth was observed, and increased apoptosis during brain development was proposed to be linked to microcrephaly in these patients (Verbeek et al. 2012). Cellular stress related to unfolded protein response increasing apoptosis in neural progenitor cells was observed in syndromic microcephaly (Poulton et al. 2011). A similar mechanism in *COL4A1/-2* mutations should be discussed, since in mutant cells, cellular stress related to unfolded protein response due to intracellular collagen accumulation was observed (Gould et al. 2007; Jeanne et al. 2012; Guiraud et al. 2017), see also Ch. 1.2.6.2.2.

4.1.1.2 Extra-CNS involvement

Particularly ocular disease and gestational abnormalities were striking features in our epilepsy patient cohort. Our data support the hypothesis that *COL4A1/-*2 mutations cause a multisystem disease due to BM impairment. However, we recommend considering an ascertainment bias leading to diagnosis of the genetic mutation only in case of multisystem impairment.

Ocular system: In all our patients, we identified abnormalities in the visual system. These included congenital cataract in 4 cases, strabismus in 3 cases, anterior chamber dysgenesis (bilateral Peters

anomaly in patient 4), coloboma in patient 8, partial bilateral nerve atrophy in patient 7, and unclear perceptive disorders (in patients 2 and 3) which were detected after birth or at infant age. Ocular disease was the most common disease after neurological system disease among epilepsy patients with *COL4A1/-2* mutations and was found in 16/55 already published and 19/38 new presented cases summarised in a recent review (Zagaglia et al. 2018). It was proposed, that anterior chamber dysgenesis in *COL4A1* may be caused by pathogenic events during early development of ocular structures, possibly involving the vasculature and the ocular lens (Mao et al. 2017). Further mechanisms supposed to cause ocular impairment in *COL4A1/-2* mutations include cellular stress due to misfolded collagen (Xia et al. 2014), vascular defects leading to retinopathy (Alavi et al. 2016), mislocalisation of radial glia cells leading to optic nerve atrophy (Labelle-Dumais et al. 2011), as well as metabolic changes involved in photoreceptor degeneration (Trouillet et al. 2017) and were introduced in section 1.2.6.2.1.

Uterine/placental impairment: Two miscarriages are reported in clinical history of the mothers carrying *COL4A1* mutations in our cohort. We suggest that this finding, together with the finding of IUGR/SGA in four of our patients, and HELLP syndrome in one mother of our cohort indicate a general impairment of the placenta, which may be both of maternal and/or fetal origin. In a mouse model of *COL4A1*, Pöschl also showed placental dysfunction (Pöschl et al. 2004). Shah et al. reported preeclampsia in family history of patients with *COL4A1* mutations (Shah et al. 2012). *COL4A1/-2* were listed as risk genes for preeclampsia (Yong et al. 2014), and maternal inheritance was found to lead to a more severe phenotype than in paternal inheritance putatively due to uterus vessels impairment (Zagaglia et al. 2018). Breaches in the BM of the Reichert´s membrane, a nonvascular placental membrane separating trophoblast and endoderm cells were reported in *COL4A1/-2* null allele mice (Pöschl et al. 2004). These findings support the hypothesis of a placental impairment in *COL4A1/-2* mutations.

Kidney disease: Renal impairment was not found in our patients, in contrast to findings in previous studies (Gale et al. 2016, Zagaglia et al. 2018), see also Ch. 1.2.2 and 1.2.6.2.1. Because of the retrospective design of this study, renal sonography was not available in all patients. Renal sonography in 3 patients did not show any abnormalities. Renal parameters were normal in blood analysis in all patients.

Other systems: Recurrent respiratory affections were found in four patients of our cohort. Even if relation to neurologic impairment and consequent aspiration events can be hypothetised (as in patient 9, showing dysphagia), pulmonary impairment in patients with *COL4A1/-2* mutations needs to be further investigated, since the BM plays an important role in alveolar system, according to previous studies: so far, one report about alveolar hemorrhage in a patient carrying a *COL4A1* mutation has been published (Abe et al. 2017). Respiratory defects were observed in mutant *COL4A1* mice (Gould et al. 2006). In a further study, *COL4A1* mutant mice showed defects in the development of the pulmonary vascular system and in the blood-gas barrier (Loscertales et al. 2016). Loscertales et al. indicated an important role of collagen IV in alveolar morphogenesis.
Because of the retrospective design of our study, no functional or histological analysis of the pulmonary system could be performed in our patients.

Creatine Kinase: Analysing potential pointers for *COL4A1/-2* related disease, high creatine kinase (CK) levels were observed sporadically in 6 out of 9 patients in our cohort. In other studies, CK has been reported as useful pointer for *COL4A1* mutation in patients with neurological disease, neuroimaging abnormalities and partially with muscle cramps (Tonduti et al. 2012). In infants and in the case of intellectual impairment, reporting of muscle cramps may be difficult. Moreover, high CK levels can be an unspecific finding, especially in the case of repeated seizures. Therefore, we suggest that CK levels are an unreliable pointer for *COL4A1/-2* mutations.

4.1.1.3 Family history

In our cohort, two inherited and four *de novo* mutations were found. In the other cases, genetic analysis of the parents was not available. Inherited mutations were associated to miscarriages in two mothers and stroke in one of them. In the three families without genetic analysis, no evident signs of probable inheritance were found.

Therefore, family history for neurological and gestational disease may be a clue for finding patients with *COL4A1/-2* mutations, but in case of unremarkable family history, patients should not be excluded from genetic testing.

Prenatal counselling may be requested by parents carrying *COL4A1/-2* mutations. Termination of pregnancies was reported depending on pathologic fetal ultrasound before *COL4A1* mutations were diagnosed (Garel et al. 2013; Colin et al. 2014, see Table *11*), and according to a recent report, embryos showing *COL4A1* mutations associated to paternal HANAC were not implanted during *in vitro fertilisation* (Patel et al. 2016). Variable penetrance and expressivity in *COL4A1/-2* mutations needs to be considered when counselling families.

4.1.1.4 Genotype-phenotype correlation

In the nine patients of this cohort, ten previously not reported mutations in *COL4A1* and *COL4A2* genes were detected. Eight mutations affected the triple helical (THX) domain. Figure *24* depicts schematically the location of mutations detected in our patients.

Glycine substitution was found in eight out of ten mutations in our cohort. Glycine mutations in THX domain have been reported to be the most common mutations in a review on all till then published 93 *COL4A1* and 10 *COL4A2* mutations (Jeanne and Gould 2017). Protein dysfunction caused by glycine mutation was predicted (Khoshnoodi et al. 2008). Although location of the mutation near the carboxyl end seemed to be related to more severe pathology for cerebrovascular disease (Jeanne and Gould 2017), data extracted in our cohort is not sufficient to confirm this hypothesis (see Figure *24*). Mutations in NC1 domain have been previously reported in patients with epilepsy and porencephaly (Yoneda et al. 2013) and in renal disease (Gale et al. 2016). The two mutations found in NC1 domain in our study (second *COL4A2* mutation in patient 2, *COL4A1* mutation in patient 5) had been classified as variants of unknown significance (VUS). Patient 2 showed a further *COL4A2* mutation in addition to the VUS, and additive effects of both *COL4A2* mutations can be suspected. It has been previously speculated on potential additive effects of mutations in the case of a patient showing two *COL4A1* mutations in two different alleles (Decio et al. 2015). In patient 5 of our cohort, a mosaicism for a supernumerary chromosome 19 was found in addition to the VUS in *COL4A1*. We speculated on the possibility of combined effects of the VUS in *COL4A1* and of the chromosomal anomaly on the epilepsy phenotype in patient 5, since malignant refractory epilepsy was described in identical twins with supernumerary ring chromosome 19 (Shahwan et al. 2004). However, while in our patient MR-changes were suggestive of a *COL4A1* mutation, in the case report of Shahwan et al. on the carriers of supernumerary ring chromosome 19 no MRI abnormalities were reported. Hence we strongly assume clinical pathogenicity of the *COL4A1* mutation in patient 5, even if combined effects of the *COL4A1* mutation and of the chromosomal anomaly are conceivable. Although the above mentioned mutations in patients 2 and 5, and *COL4A1* mutation in patient 7 were classified as VUS, we find it plausible to assess them as disease-causing, since a typical pattern of clinical and neuroimaging findings suggestive of a *COL4A1/-2* mutation could be found in these patients.

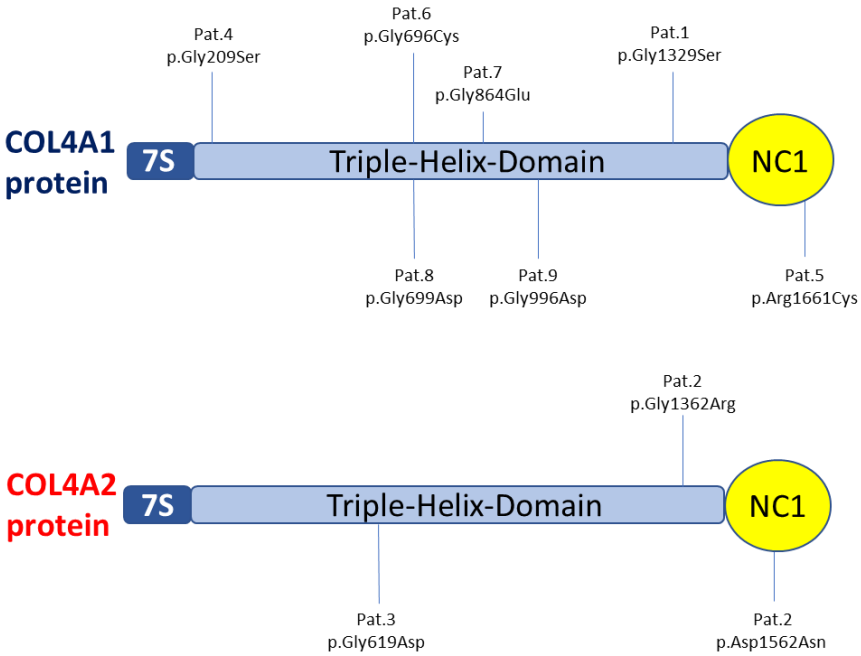


Figure 24. COL4A1 and -2 protein, functional domains: distribution of mutations in patients 1-9.

A milder phenotype in patients with *COL4A2* mutations was proposed, even if an ascertainment bias leading to more frequent recognition of patients with *COL4A1* than *COL4A2* mutations was discussed (Jeanne and Gould 2017). In our cohort, two patients with *COL4A2* mutations showing very severe epilepsy were found, so that the hypothesis of a milder impairment in patients carrying *COL4A2* mutations cannot be confirmed.

Since relation between phenotype and genotype is still not profoundly understood, we propose that possible pathogenicity should be accurately evaluated also in cases of unclear genetic significance of *COL4A1/-2* mutations.

4.1.2 What are typical neuroimaging findings?

MR imaging can help in the identification of patients with *COL4A1/-2* mutations as a part of screening protocol. Moreover, it can provide hints for pathophysiology in affected patients.
In our cohort a broad spectrum of findings was observed (see Table *8*), including cortical abnormalities, bihemispherical white matter change (WMC), periventricular leukomalacia (PVL), ventriculomegaly and cerebellar microbleeds. Asymmetry in cerebral volume of the hemispheres or signs of past hemorrhage/infarction were found. Also defects in basal ganglia and thalamus, as well as hippocampal pathologies were present.
Most importantly, in all our patients cortical abnormalities of various degree were detected, supporting our hypothesis that cortical malformations play an important role in *COL4A1/-2* epilepsy patients.
In a recent review, white matter change, porencephaly, cerebral calcifications, microbleeds and intracerebral hemorrhages were the most frequent neuroimaging findings among 152 previously published and 21 newly presented patients harboring *COL4A1/-2* mutations (Meuwissen et al. 2015). Different clinical features of the examined cohorts should be considered when comparing results of Meuwissen et al. to results in our cohort. While patients reported in the review of Meuwissen et al. showed various clinical phenotypes, severe drug-resistant epilepsy was the main clinical feature and inclusion criterion for the study on our patients and may explain the finding of cortical abnormalities in all our patients.

4.1.2.1 Cortical abnormalities

Cortical abnormalities were found in all patients of our pediatric epilepsy cohort**.** Neuroimaging revealed multiple cortical abnormalities (patients 1-3 and 8), findings typical for FCD Type I (patients 5-6), polymicrogyria and schizencephaly (patients 4 and 7) and changes resembling pachygyria (patient 9), see Table *7* and Table *8*.

Cortical malformations related to *COL4A1* or *-2* mutations were observed in MRI in 32 patients identified in our literature review. Schizencephaly was observed most frequently among reported cortical malformations (see Table *10*). It is remarkable that 26 out of 32 patients showed epilepsy or seizures.

Importantly, the histological analysis confirmed MCDs in our patients 1-3 (FCD Type III, mMCD, heterotopias, see Table 4 and Ch. 4.4.1.1).

In agreement with our observations, the relevance of cortical abnormalities in epilepsy patients carrying *COL4A1/-2* mutations was underlined in a recent review: MCDs including schizencephaly, polymicrogyria, focal cortical dysplasia and nodular heterotopias were identified in neuroimaging of 7/55 previously published and 11/38 newly presented cases (Zagaglia et al. 2018).

Therefore, the findings from our patients and our literature review support the relevance of cortical changes in *COL4A1/-2* associated epilepsy.

Pathophysiology leading to these changes is still partly unclear, but consistently with our histological findings, impairment of cortical development, either due to punctual vascular pathology, or to breaches in the pial basement membrane during neuronal migration may be underlying, as observed in previous animal studies (Pöschl et al. 2004, Favor et al. 2007), see also Ch. 4.4.1.1.

4.1.2.2 White matter change

Periventricular and deep WMC was observed in all our patients (see *Table 8*). Corpus callosum thinning or hypoplasia was observed in six out of nine patients, unilateral brain stem thinning in patients 1, 3 and 6. In patients 6 and 8, signs for Wallerian degeneration were found.

Moreover, unilateral or bilateral ventriculomegaly was found in all patients of our cohort.

Poor white matter development can be explained with hypoxia in progenitor cells resulting in hypomyelination during cerebral development, but also disturbed oligodendrocyte maturation and neuroinflammation may play a role in white matter impairment (Tolcos et al. 2017). PVL was defined as a "posthypoxic-ischemic leukencephalopathy resulting from a pre- or perinatal hypoxic-ischemic insult" (Meuwissen et al. 2015)", and is associated with ventriculomegaly through lateral dilatation of the ventricle following white matter atrophy. Thus, it is conceivable that hypoxic states during gestation may have contributed to the detected white matter changes in our patients.

Only in patient 8 of our cohort a cystic defect similar to a porencephalic cyst[13] was detected in neuroimaging, see Table *7*. Interestingly, in patient 9 of our cohort, a cystic defect communicating with the left ventricle was also found but was defined as ventricular enlargement (see Table *7*). Different definitions of cystic defects may be an obstacle for the identification of patients with *COL4A1/-2* mutations.

Pathology underlying cystic encephalomalacia involves late gestational, perinatal or neonatal brain injury, leading to liquefaction necrosis (Barkovich and Raybaud 2012, Ch.4). It is conceivable that the cystic defects detected in neuroimaging in patients 8 and 9 are caused by similar pathogenic mechanisms.

White matter change was frequently observed in previous studies on patients with *COL4A1/-2*

[13]Porencephaly has been defined differently in the last decades. In this study, porencephaly is defined as a defect following a focal hemorrhagic necrosis. This defect is usually transhemispheric, communicates with cerebrospinal fluid system and extends to the cortical layers. It does not normally follow a venous infarction, whose localisation is different and originates in the germinal matrix (as defined by Prof. Peter Winkler, 2017). Radiologic findings, which can correspond to ventriculomegaly have been defined as porencephaly in other articles (Meuwissen et al. 2015).

mutations: adult-onset leukencephalopathy including porencephalic cavities (Ayrignac et al. 2015), porencephalic cysts, ventriculomegaly and diffuse PVL (Meuwissen et al. 2015; Zagaglia et al. 2018) were among the most common neuroimaging findings detected in patients carrying *COL4A1/-2* mutations. In the reports about epileptic surgery in patients with *COL4A1* mutations, atrophy of periventricular white matter, thinning of corpus callosum (Papandreou et al. 2014) ventriculomegaly and increased periventricular enhancement were described (Hino-Fukuyo et al. 2017), as shown in Table *9*.
Thus, consistently with the observations from the above mentioned previous studies, our results confirm that white matter change is an important diagnostic feature in patients harboring *COL4A1/-2* mutations.

4.1.2.3 Microbleeds and germinal matrix hemorrhage

Changes suggestive of disseminated microbleeds, especially in cerebellum, were observed in patients 1, 2 and 4, see *Table 8* and *Figure 13*. Typical timing for cerebellar hemorrhage is the fetal period, because of the highly vascularized cerebellar germinal matrix (Barkovich and Raybaud 2012, Ch.4). Vessel fragility due to abnormal collagen IV was proposed as putative mechanism for hemorrage and small-vessel disease in patients with *COL4A1* mutations (Gould et al. 2006). Thus we suggest that mechanical instability of vessels during cerebellar fetal development is likely to be the cause of cerebellar microbleeds in patients with *COL4A1/-2* mutations.
Defects of the basal ganglia or of the thalamus were observed in six patients in our cohort, and according to Barkovich and Raybaud, they can give hints for venous infarction occurred during fetal development (Barkovich and Raybaud 2012, Ch.4).
In patients 8 and 3, germinal matrix hemorrhages were suspected (see *Figure 21*).). The caudothalamic groove (the pit between caudate nucleus and thalamus) is a typical location for subependymal germinal matrix hemorrhages in fetuses (Barkovich and Raybaud 2012, Ch.4).
We propose that both cerebellar and subependymal defects in the germinal matrix in our cohort can be explained with vessel instability due to impairment in vascular basement membrane.

4.1.2.4 Hippocampal defects

Hippocampal pathology, including hippocampal sclerosis, atrophy and malrotation was observed in four patients in our cohort (see *Table 8*). Hippocampal sclerosis in patient 1 was confirmed in histological analysis, see section 4.4.1.2. To our knowledge, this is the first study reporting hippocampal sclerosis related to *COL4A1/-2* mutation in patients. In *COL4A1* mutant mice, defects in hippocampal layers and hippocampal gliosis were reported in one article (Labelle-Dumais et al. 2011). Further studies are needed to investigate the association of *COL4A1/-2* mutations with hippocampal defects, as well as its clinical and therapeutical consequences.

4.1.2.5 Cerebral calcifications

Cerebral calcifications were not found in our cohort, except for putative findings in patient 4. In other studies, finding of cerebral calcifications together with PVL was reported as possible pattern for recognizing patients with *COL4A1* mutations (Livingston et al. 2011). Calcification can be generally found after hemorrhagic, ischemic or infectious cerebral damage, but detection of calcification in association with PVL is otherwise unusual. When interpreting our findings, it needs to be considered that detection of calcification might be complex: even in computed tomography (CT), calcification is not surely distinguishable from hemorrhage, and MRI sequences are less reliable than CT imaging, since calcium and iron show similar magnetic properties (Livingston et al. 2014).
In children, CT imaging is often not performed to avoid side-effects of radiation. In our patients, CT imaging was not performed, thus we cannot exclude undetected calcifications in our cohort.

4.1.3 Timing of brain injury: at which developmental stages are lesions observed?

In literature, intrauterine (Durrani-Kolarik et al. 2017), neonatal (Kellett et al. 2018), childhood (Shah et al. 2010) and adult-onset stroke (Gould et al. 2005; Gould et al. 2006; van der Knaap et al. 2006) associated to *COL4A1* mutations were reported. In our cohort of pediatric epilepsy patients, no childhood stroke was observed, but prenatal brain injury was suspected in 8 out of 9 patients (all patients except for patient 5).

In all patients of our cohort who underwent surgery, prenatal lesion was suggested. In patient 1, imaging at six months age raised the suspicion of a prenatal infarction. In patient 2, bilateral intraventricular hemorrhage was found after birth and SWI-imaging at five months age showed multiple susceptibility defects and a bigger hemorrhage. In patient 3, a subependymal germinal matrix hemorrage in fetal time was suspected. Prenatally observed ventriculomegaly was found in patient 4 and patient 7. In patient 8, a postnatal ultrasound showed signs of a subependymal bleed. In patient 6, a prenatal infarction was suggested as causative for the observed pathology in neuroimaging. Moreover, the cortical abnormalities observed in the other patients may have been caused by a prenatal vascular injury. In this case, all patients of our cohort would be showing a neurological phenotype following prenatal brain injury.
Our observations suggest that prenatal brain injury may play an important role for impairment of cortical development and epilepsy phenotype in patients harboring *COL4A1/-2* mutations. To further support this hypothesis, we performed a literature review identifying 12 articles reporting in total 17 carriers of *COL4A1* mutations in which brain injuries were documented in the prenatal period, as shown in *Table 11*. According to our literature review, earliest finding of increased echogenicity was documented in the 19th gestation week, i.e. in the fifth gestational month, and pathological changes were mostly observed between the 20th-30th gestational weeks in patients carrying *COL4A1* mutations. The peak time period for neuronal migration in humans is from the 3rd to 5th gestational month, while the peak time period for neuronal organisation begins in the 5th gestational month (Volpe 2018, Ch.6-7). In *Figure 25*, we propose a model for the timing of *COL4A1*-associated prenatal brain injury in relation to the development of the human cortex.

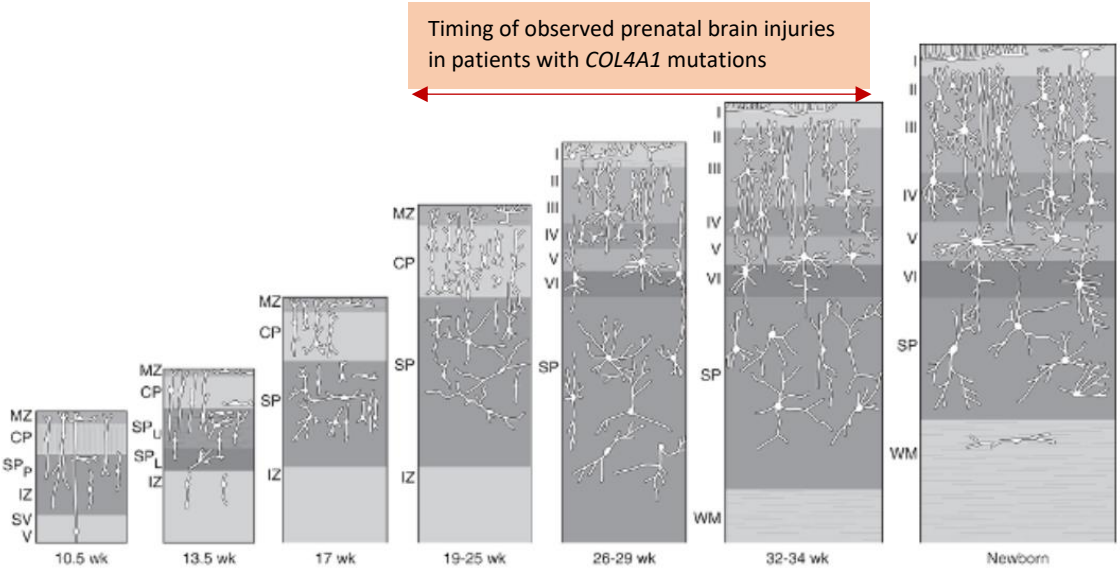


Figure 25. Timing of *COL4A1*-associated prenatal brain injury in relation to development of human prefrontal cortex. Adapted from (Volpe 2018), with permission from the editor. Based on literature review presented in Table 11. CP: cortical plate, IZ: intermediate zone, MZ: marginal zone, SP_L: subplate zone (lower), SP_p: subplate-preplate zone, SP_u: subplate zone (upper), SV: subventricular zone, V: ventricular zone, wk: week of age, WM: white matter, I-VI: cortical layers.

To our knowledge, no studies documented the occurrence of cerebral injury in the prenatal period in patients harboring *COL4A2* mutations. However, the case of patient 2 in our cohort, presenting two

COL4A2 mutations and residuals of prenatal brain injury, suggests that similarly to *COL4A1* mutations, also *COL4A2* mutations are related to pre/perinatal brain injury.

Thus, on the basis of suspected prenatal brain injury in our patients and of our literature review, we suggest that impaired cortical development in patients with *COL4A1/-2* mutations can be explained by fetal brain injuries that occurred in the time period of late neuronal migration and cortical organisation. Hereby we support the observation of Khalid et al., who described schizencephaly as the consequence of a vascular event occurred in the 19th GW in a patient carrying a *COL4A1* mutation (Khalid et al. 2018), see also Table *11*.

In 8 out of the 17 *COL4A1* cases found in our literature review (see Table *11*), prenatal brain injuries were related to infant epilepsy or seizures. This observation, supported by results from neuroimaging studies in our patients and from our review on MCDs in further patients with *COL4A1/-2* mutations, leads us to hypothetise that the development of epilepsy may be related to the degree of cortical affection in patients harboring *COL4A1/-2* mutations. Further studies are needed to confirm this hypothesis.

Based on findings in rodents, it was proposed that caesarean delivery may protect patients with *COL4A1* mutations from severe intracerebral hemorrhages (Gould et al. 2006). Besides the fact that differences in gestation and parturition between rodents and humans should be considered, it is remarkable that in six out of nine patients of our cohort, brain pathologies were found despite caesarean delivery. Cerebral pathologies seemed not to be more severe in patients after vaginal delivery (patients 5,7,8) if compared to the other six patients of our cohort. On the basis of these observations, we suspect that prenatal pathologies were the main cause of brain injury in our patients. However, it cannot be excluded that increased intracranial pressure during vaginal delivery could further aggravate CNS phenotype in patients carrying *COL4A1/-2* mutations. Thus, caesarean delivery may represent an important precaution in peripartal management if fetal or maternal *COL4A1/-2* mutation is known.

4.1.4 Which diagnostic clues can be found in *COL4A1/-2* epilepsy patients?

According to findings in our cohort, clinical diagnostic clues suggestive of a COL4A1/-2 mutation in children can be severe early-onset epilepsy, motor dysfunction, developmental delay and microcephaly as well as ocular impairment.
In all patients of our cohort, bilateral pathology affecting gray and white matter was observed in neuroimaging. Importantly, cortical pathologies were detected in all patients (see Table 8).
Suspected pathology underlying MRI changes in our patients included past vascular insults, hypoxic-ischemic states, and disturbances in neuronal migration and cortical organisation, leading to a broad pattern of MRI findings including MCDs, white matter change and cerebellar microbleeds.
We propose that depending on the timing and location of cerebral injury, different patterns of MRI abnormalities can be expected in patients with *COL4A1/-2* mutations.
According to our results the finding of multiple lesions, dating to different developmental stages of the brain, is also a diagnostic clue for *COL4A1/-2* mutations in patients. Our results indicate that even if microbleeds or major hemorrhages are absent, mild cortical or white matter abnormalities together with clinical history of epilepsy and developmental delay can give a hint for a potential *COL4A1/-2* mutation.

4.2 How can we define candidacy for epilepsy surgery in *COL4A1/-2* related epilepsy?

COL4A1/-2 mutations can lead to a genetic epilepsy, which clearly shows structural abnormalities, as observed in our cohort and in previous studies (Meuwissen et al. 2015, Zagaglia et al. 2018).
We did not identify particular features defining candidacy for epilepsy surgery which are solely specific to patients with C*OL4A1/-2* mutations. Nevertheless several aspects that could be derived from clinical history of our patients and from our literature review on epilepsy surgery in patients carrying *COL4A1/-2* mutations can be valuable for evaluating surgical candidacy and will be discussed in the following.
In our study one patient with *COL4A1* (patient 1) and two patients with *COL4A2* mutations (patients 2 and 3) showing severe childhood drug-resistant epilepsy underwent surgical procedures. In these patients a clear epileptogenic focus or a epileptogenic hemisphere was found in presurgical evaluation and a resective or disconnective surgery was performed.
Patients with resectable or disconnectable epileptogenic areas can profit from surgery, as shown in patient 3, that had a seizure free-outcome (Engel Ia), but also in patients 1 and 2, where a reduction in seizure frequency was achieved (Engel IIIa), see also Table 5.
Palliative surgical procedures may help patients with severe or encephalopathic course of epilepsy: enabling developmental steps at infantile age and stopping epileptic encephalopathy was the aim of surgery in patient 2, showing bilateral multifocal epilepsy with right-sided maximum.
In the other six patients of our study showing *COL4A1* mutations and severe drug-resistant epilepsy (see also Table *6*), presurgical evaluation showed multiple bilateral foci (patients 7 and 8) or missing concordance of abnormalities in EEG and MRI (patients 4 and 5), leading to decision against surgical intervention. In one case, on the basis of EEG and MRI findings , the chance for seizure freedom after surgery was expected to be very low and led to further conservative treatment (patient 9). In patient 6, sufficient seizure control with AED was the reason for continuation of conservative treatment. Unfortunately, clinical course was not favourable in the four out of the six patients (patient 4,5,8,9) with available follow-up (min 4 months, max 10 years after presurgical evaluation).

In three cases epilepsy surgery has been reported in *COL4A1* related epilepsy so far (see our review of literature in Table *9*). In the first reported case, “severity of epilepsy and its major negative impact on quality of life” was the reason leading to the decision to offer epilepsy surgery, even if increased surgical risk of hemorrhage was suspected in patients with *COL4A1* mutations (Papandreou et al. 2014). Importantly, in the reported cases and in our patients´cohort, no perioperative complications such as stroke or hemorrhage, in contrast to previous speculations because of Collagen IV impairment (Papandreou et al. 2014; Hino-Fukuyo et al. 2017), were observed.
Interestingly, in one further reported case (Hino-Fukuyo et al. 2017) and in patient 1 of our study, patients underwent epilepsy surgery twice. At the time of the first surgical procedure, *COL4A1* mutation was not known in our patient. In the patient reported by Hino-Fukuyo et al., a *COL4A1* mutation was diagnosed after the second surgical procedure, thus the decision to operate was made independently of the genetic status. In patient 1 of our study, a second procedure consisting of a left-sided hemispherotomy was performed because of severe impairment of daily life by drop seizures and left-sided MRI- and EEG-abnormalities.

Because of the very variable phenotype in *COL4A1/-2* mutations, epilepsy surgery should be evaluated considering individual patients´ history in specialised centers. Similarly to presurgical evaluation in other cases of drug-resistant epilepsy, depending on epileptogenic foci, adjacent eloquent areas, age of the patient and burden of epilepsy, candidacy for a curative or palliative surgical procedure can be discussed.

4.3 What is the outcome of epilepsy surgery in patients with *COL4A1/-2* mutations?

In our study, patient 1 underwent lesionectomy and hemispherotomy, patient 2 lesionectomy and disconnection, and patient 3 hemispherotomy. In patient 2, surgery was performed with a clear palliative aim, and in both patients 1 and 3, chance of seizure freedom after surgery was expected to be low.
One out of three patients showed seizure-free outcome (Engel outcome Ia in patient 3, 6 months after intervention), two out of three patients showed worthwhile seizure reduction (Engel outcome IIIa in patients 1 and 2, 12 and 24 months after intervention respectively), see *Table 5*.
Neurocognitive improvement was observed in patients 1 and 2 despite persisting seizures.

As described in the introduction, outcome of epilepsy surgery in terms of seizure-freedom is generally very positive, but less favorable in case of genetic epilepsy. In the case of epilepsy surgery for MCDs, which were detected in our patients, rates of seizure-free outcome vary in literature, see reported studies in Ch. 1.4.4.3 .
Three cases of epilepsy surgery in patients carrying *COL4A1* mutations with seizure-free outcome for several months have been reported so far (Papandreou et al. 2014; Hino-Fukuyo et al. 2017; Zagaglia et al. 2018), see Table *9* in Chapter 3.2.
Findings in our cohort and in literature review indicate that based on the different phenotypes, varying outcome of epilepsy surgery is to be expected. Very severe epilepsy, such as in patient 2, may be very difficult to be controlled either with epilepsy surgery or with AEDs.
However, in case of a very severe drug-resistant *COL4A1/-2* epilepsy, outcome should be not only considered in terms of seizure-freedom, but also in terms of general quality of life and cognitive improvement.

In summary, we pointed out that epilepsy surgery can lead to decreased seizure frequencies in selected patients carrying *COL4A1/-2* mutations. Even if seizure freedom cannot be achieved in all patients, the surgical option may nevertheless enable developmental steps and should be taken into consideration.

4.4 What is the pathophysiology underlying *COL4A1/-2* related epilepsy?

4.4.1 Histological findings: Which clues about pathophysiology can we find?

Our study evaluated brain tissue specimens in one *COL4A1* and two *COL4A2* pediatric epilepsy surgery patients.
In all three patients cortical pathologies were observed and in patient 1, hippocampal sclerosis was found (see *Table 4*).

4.4.1.1 Malformations of cortical development

In patient 1, FCD associated to a cystic defect and hippocampal sclerosis was observed, see *Figure 6-9*. Cortical dysplasia can be caused by a vascular insult during cortical development, as observed in a previous study, in which 12.5% of histologically confirmed MCDs were related to vascular injury (Krsek et al. 2010). Thus, past vascular injury may explain findings in patient 1 of our cohort.
In patient 2, mMCD type II was diagnosed, see *Figure 17*. No bleeding residuals or direct brain injury correlates were found. Another possible mechanism leading to cortical dysplasia in the absence of brain injury, is disturbance of neural migration related to pial BM changes: Pöschl et al. showed neuronal ectopias in the histology of a mouse model with deficient *COL4A1/-2* locus and stated that disruption of the pial basement membrane is the most probable reason for aberrant neural cells (Pöschl et al. 2004). Favor et al. also observed defects reflecting neuronal migration disturbances in *COL4A2* mice (Favor et al. 2007), see also Attachments, Chapter 9.4.

In patients 1-3, white matter heterotopic neurons were found (see *Figure 8, Figure 9, Figure 18 and Figure 22*a - b). Additionally, in patient 1 periventricular nodular and lentiform heterotopias were found. Heterotopia can be defined as "accumulation of normal-appearing neurons in abnormal locations" (Barkovich et al. 2015). Discussed pathophysiology underlying periventricular heterotopia includes not started neuronal migration from ventricular zone to the cortex and defects in neuroependyma, leading to disturbed attachements of neurons to radial glia during migration (Fox and Walsh 1999; Barkovich et al. 2015: see 9.4, in which a schematic model proposed by Barkovich et al. is depicted). Neuroependymal BM underlying choroid plexus in mice was found to contain α1 and α2 chains, together with α3α4α5 collagen chains (Urabe et al. 2002). Thus, we propose that disruption of neuroependymal BM is conceivable in patients with *COL4A1/-2* mutations.
Ectopias were observed in mice due to breaches in pial BM, and the authors predicted that "that any mutation that affects pial basement membrane assembly or stability leads to cortical dysplasia"(Halfter et al. 2002, see 9.4 for a schematic model proposed by the authors).
The finding of heterotopias in the post-mortem histology in two neonates carrying *COL4A1* mutations (Colin et al. 2014) and in neuroimaging of patients with *COL4A1/-2* mutations (Tonduti et al. 2012; Cavallin et al. 2018; Zagaglia et al. 2018), together with the mentioned findings from animal studies, corroborates the hypothesis that neuronal migration is impaired due to *COL4A1/-2* mutations.

Remarkably, the importance of the histological finding of MCDs in patients who underwent surgery is reinforced by the detection of MCDs of various degree including schizencephaly and polymicrogyria in neuroimaging in the patients who did not undergo epilepsy surgery (patients 4-9) (see *Table 7* and *Table 8*).

4.4.1.2 Hippocampal sclerosis

In patient 1, hippocampal sclerosis was observed (see *Figure 10*). Hippocampal defects were observed in neuroimaging in four patients of our cohort (see 4.1.2.4). To our knowledge, no hippocampal sclerosis has been reported previously in patients with *COL4A1/-2* mutations, but hippocampal defects have been reported in a *COL4A1* mutant mice model for WWS/MEB (Labelle-Dumais et al. 2011).
We suggest that in patient 1 of our cohort, past brain injury as well as the severe epilepsy may be causative of impaired development of the hippocampal architecture.

4.4.1.3 Other findings

Gliosis was detected in histological specimens of our patients 1-3. In patient 1, gliosis was observed adjacent to the pseudocyst corresponding to an old damage (see *Figure 6*). The ability of the brain to create an astrocytic response to injury begins in the late second or third trimester of pregnancy (Barkovich and Raybaud 2012, Ch.4). Thus, a brain injury dating to this time or later is probable in patient 1. In patient 2 and 3, gliosis was observed, but no pathologic correlate of an old damage was found. Moreover, in patient 2 hypomyelination and in patient 3 demyelination were described. In patient 3, past prenatal injury was proposed as causative for gliosis and demyelination. These findings correlate with white matter change found in neuroimaging.
In patients 1 and 2 of our cohort, focally increased perivascular lymphocytic infiltrates were found. Regulation of immune cells diapedesis in CNS involves interaction with BM structures and signalling (Sorokin 2010).
However, clinical findings available in our cohort did not provide evident clues for an immune-caused pathology related to *COL4A1/-2* mutations. Further studies are needed to clarify the role of immune cells in *COL4A1/-2* pathology.

4.4.1.4 Histological clues for understanding pathophysiology in *COL4A1/-2* associated epilepsy

To date, only a few human histologies have been described in *COL4A1* mutations. In one case, FCD Ia was diagnosed, based on cortical architectural abnormalities, neuronal clustering and neurons found in subcortical white matter (Yoneda et al. 2013), see also Table *10* and Chapter 3.2.2. In post-mortem histology of two neonates, hemorrhagic lesions and heterotopias were respectively found (Colin et al. 2014), see also Table *11* and Chapter 3.2.3. In a patient carrying a *COL4A1* mutation with cortical dysplasia in MRI, cerebral blood vessels showed homogenous thickness of COL4A1 protein in the patient and in the control case and more pronounced small vessel permeability in the patient with the *COL4A1* mutation than in the control case (Zagaglia et al. 2018).
Thus, on the basis of current literature, there is only scarce knowledge on histology in patients with *COL4A1* mutations so far.
In patient 1 of our cohort (carrying a *COL4A1* mutation) FCD putatively associated to a past vascular insult was found, while in patient 2 and 3 of our cohort (carrying *COL4A2* mutations) we detected mild malformations of cortical development without the finding of associated past bleeding residuals.
Together with the limited data on human histology reported in literature, our results suggest that both hemorrhagic lesions, as well as cortical dysplastic changes, which not seem to be solely related to vascular lesions, are typical findings in patients carrying *COL4A1/-2* mutations. We speculate that in addition to vascular injury, a plausible mechanism for the manifestation of cortical dysplasia in patients harboring *COL4A1/-2* mutations is the disturbance of neuronal migration consecutive to pial BM changes, as proposed in animal studies (Pöschl et al. 2004; Favor et al. 2007).

Importantly, we need to consider that our histologic findings are limited to resected areas. In patients 2 and 3, due to the disconnective surgical procedure only limited specimens were available and important clues for a complete understanding could be missing. Moreover, changes secondary to epilepsy should be considered.

4.4.2 What are possible mechanisms of epileptogenesis?

In this section we discuss different epileptogenic mechanisms and propose a schematic model for epileptogenesis in *COL4A1/-2* mutations (see *Figure 26)*.

We suggest that epileptogenesis in patients with *COL4A1/-2* mutations may be due to blood products remaining after hemorrhages, consistently with diffuse microbleeds following vascular instability as found in patient 2, see also susceptibility weighted MRI in Figure *13*. Epileptogenicity of blood products containing iron has been demostrated in several animal models (Ueda et al. 1998; Rosen A. D. and Frumin N. V. 1979). Correlation of seizures with hemorrhagic infarctions in tumors also support this argument (Roelcke et al. 2013).
However, in specimens from epileptogenic areas of patients 2 and 3, no signs of past bleeding, in terms of iron products in Prussian blue staining were found. Thus we assume that further mechanisms play a role in epileptogenesis associated with *COL4A1/-2* mutations.

We speculate that epileptogenesis associated with *COL4A1/-2* mutations could be due to impairment of cortical architecture and neuronal ectopias. MCDs were detected in our patients through imaging (in pat. 1-9, see Table *8*) and histological analysis (in pat. 1-3, see *Table 4*). Our literature review on *COL4A1/-2* epilepsy patients supported the importance of the finding of MCDs in *COL4A1/-2* related epilepsy (see Table *10*). Malformations of cortical development and FCD have been associated with epilepsy, even if the pathogenetic mechanism is still unclear (Battaglia et al. 2013). Heterotopic neurons may be involved in complex epileptogenic networks (Barkovich et al. 2015). Current knowledge on epileptogenesis in MCDs was introduced in section 1.5.4.

In addition, diffuse disturbances in electrophysiological processes, such as lowering the threshold for seizure generation, which are not related to structural changes were hypothetised in *COL4A1* mutations (John et al. 2015), but no mechanism for electrophysiological change has been found yet. EEG-foci were observed also in areas without MRI-changes in our cohort (in patients 4 and 5), and in patient 3, no severe MCD could be diagnosed in histological specimens. This could indicate a general and subtler network impairment.
However it needs to be noted, that MRI resolution is not able to show mild structural changes affecting network organisation, such as heterotopic neurons, and that histologic analysis may have not provided conclusive diagnostic evidence due to limited resected specimens in our patients 2-3. Thus, further investigations are needed to examine potential electrophysiological changes related to *COL4A1/-2* mutations in cerebral tissue.

Further, it should be considered that white matter change itself could lead to changed function of cortical matter. A correlation between white matter change and cortical atrophy was found in a prospective study about patients with Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) showing that subcortical infarctions are followed by cortical thinning and neurodegeneration (Duering et al. 2012). This was discussed as relevant in the condition of post-stroke epilepsy (Tanaka and Ihara 2017). In patients of our study, diffuse white matter change, periventricular leukomalacia in MRI, gliosis, hypomyelination as well as white and gray matter volumen reduction in histological specimens were observed. Thus, epileptogenesis in patients with *COL4A1/2* mutations may be related to general network impairment after hypoxic or hemorrhagic injury and consequent diffuse defects in surrounding gray and white matter.

Intrinsic epileptogenicity of mutated COL4A1 and -2 protein is conceivable. Mutation of other BM components was shown to influence epileptogenicity: in nidogen1-knockout mice abnormal behaviour and increased hippocampal neuronal excitability were demonstrated (Vasudevan et al. 2010). Blood-brain barrier (BBB) function appeared to be normal, and defects in cortical lamination of hippocampus were not found in the study of Vasudevan et al. 2010. The authors discussed

possible function of nidogen in cell signalling. This would imply nidogen is present in hippocampal parenchyma outside of BM (Vasudevan et al. 2010). Anyway, collagen IV appears to be found in CNS only in the BM (Urabe et al. 2002), and no studies showing intrinsic epileptogenicity of COL4A1/-2 mutant proteins were found to date.

Metabolic damage to neurons and related epilepsy could be discussed as consequence of BBB impairment. Trouillet et al. investigated metabolic damage to neurons of the retina in a *COL4A1* mouse model for HANAC (Trouillet et al. 2017). Relevance of BM as a component of the BBB has been shown in research about neuroinflammation (Engelhardt and Sorokin 2009; Sorokin 2010), and effect of neuroinflammation on epileptogenesis has been discussed (Webster et al. 2017). In children with chronic epilepsies, histological damage of the BBB with splitting of the parenchymal and endothelial BM layer was found, showing relevance of white-matter angiopathy in epileptogenesis (Hildebrandt et al. 2008). In two patients of our cohort, focally increased perivascular lymphomonocytes were observed, but clinical impairment of BBB was not observed.

In conclusion, we propose that epileptogenesis associated with *COL4A1/-2* mutations results from multiple factors. Probably, morphological and network changes after vascular insults as well as fundamental defects in cortical development during the fetal period play the major role. For a schematic illustration of the here proposed epileptogenetic mechanisms see *Figure 26*.

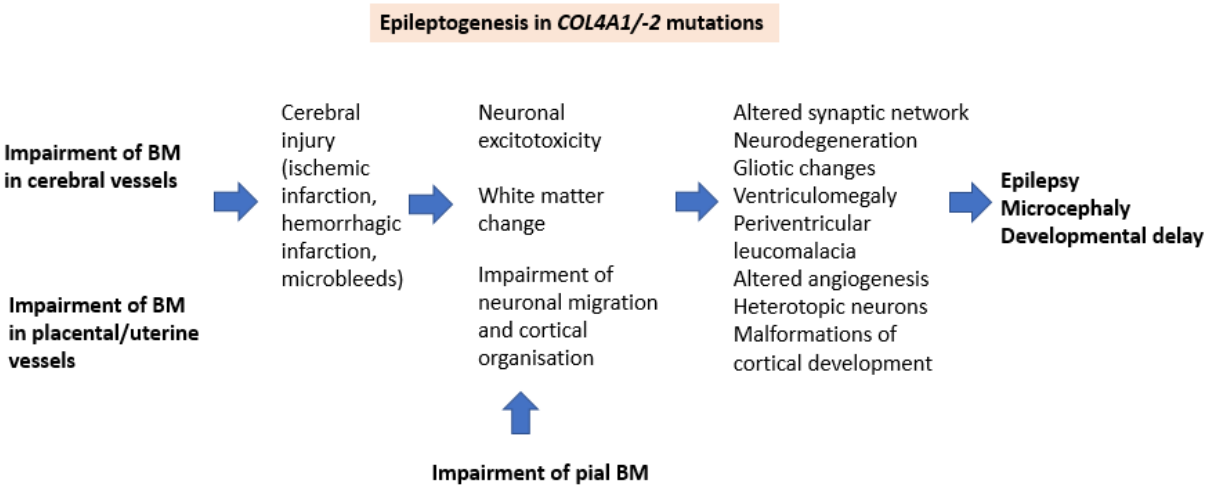


Figure 26. Proposed model of epileptogenesis in *COL4A1/-2* mutations.
Depending on timing (e.g. impaired neuronal migration in fetal cerebral development, or childhood/adult stroke), location and severity of the cerebral injury, variable clinical courses are to be expected in *COL4A1/-2* mutations. Abbreviations: BM= basement membrane.

4.5 Limitations and strengths of this study

Due to the rareness of *COL4A1/-2* mutations and consequently to the very small sample size no statistical analysis could be performed. Thus, statistical significance of findings cannot be proven. Moreover, histological findings are limited to resected specimens, in two cases (patients 2 and 3) not deriving from the principal lesion. Furthermore, due to the retrospective design of the study, only limited data is available.

It should be considered that animal models for human epilepsy and CNS pathology only provide partial explanation for clinical phenotype in patients. Our explorative-retrospective study, in which nine patients´ history and findings were analysed, provided important insights in phenotype, pathophysiology and timing of lesion in *COL4A1/-2* mutation related epilepsy.

This was the first study which extensively presented and evaluated human histology in *COL4A1/-2* mutation related epilepsy. Also, for the first time, patients with *COL4A2* mutations undergoing epilepsy surgery were presented. In patients who did not undergo surgery, reasons for conservative treatment were provided, that can be useful when considering treatment options. Family history and follow-up over several years could be provided in several cases and can help in patient counselling. Clinical management and therapeutic options were presented and evaluated. A better awareness and understanding of this disease were achieved and instruments for making diagnostic and therapeutic decisions were offered. This will help in diagnosis and analysis of bigger patient cohorts in future and in finding evidence for treatment options.

4.6 Conclusion and outlook

In conclusion, *COL4A1/-2* mutations are a relevant entity in childhood epilepsy and developmental delay. Family inheritance may be present, but in this cohort *de novo* mutations were more frequent. Based on our findings, we recommend that genetic testing for *COL4A1/-2* mutations should be performed in patients with drug-resistant epilepsy and brain lesions with putative prenatal origin, as well as in patients showing drug-resistant epilepsy, motor dysfunction, developmental delay, microcephaly and disorders of the ocular system.

Importantly, in all patients of our cohort, cortical abnormalities of various degree were detected in neuroimaging. Histological analysis confirmed cortical abnormalities including focal cortical dysplasia type IIId, mild malformations of cortical development and heterotopias in the resected specimens of the three patients who underwent epilepsy surgery.

As shown in this study, the brain injury pattern varies depending on timing and location of the vascular insult. Together with findings in our cohort, evidence from literature suggests the association of vascular and pial BM defects due to *COL4A1/-2* mutations with the impairment of neuronal migration and organisation. These defects may lead to diffuse mild cortical developmental pathologies and may play an important role in *COL4A1/-2* associated epilepsy. Thus, MRI findings in patients with *COL4A1/-2* mutations may be mild and importantly, bilateral pathology is to be expected.

According to our findings in patients with *COL4A1/-2* mutations, if there is a clear electro-clinico-anatomical correlation between an epileptogenic lesion and the ictal onset zone, epilepsy surgery may be discussed. Our observations indicate that epilepsy surgery can lead to decreased seizure frequencies, but seems to have low chance of seizure-free outcome. However it can diminish seizure severity and enable neurocognitive developmental steps achieving a reduction in burden of disease in highly selected patients carrying *COL4A1/-2* mutations.

Our study provided important insights for the understanding of *COL4A1/-2* associated epilepsy and for the diagnostic and therapeutic approach in *COL4A1/-2* epilepsy patients. Further studies will be needed for deeper understanding of pathophysiology and evaluation of therapeutic strategies.

5 Index of Tables

6 Index of Figures

7 Abbreviations

For abbreviations used in tables, please see the legend underneath the tables.

ACMG: American College of Medical Genetics
AED: antiepileptic drugs
APGAR score: Appearance, Pulse, Grimace, Activity and Respiration score
BBB: blood-brain barrier
BM: basement membrane
CADASIL: Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy
Ch.: chapter
CK: creatine kinase
CNS: central nervous system
COL4A1/-2: *COL4A1/COL4A2* gene; COL4A1/-2: COL4A1/2 protein
CT: computed tomography
d: day
ECoG: electrocorticography
EEG: electroencephalography
ER: endoplasmic reticulum
EZ: epileptogenic zone
FCD: focal cortical dysplasia
FLAIR: fluid-attenuated inversion recovery
fMRI: functional magnetic resonance imaging
GFAP: glial fibrillar acidic protein
GM: gray matter
GMFCS: gross motor functions classification system
GW: gestational week
HANAC: hereditary angiopathy, nephropathy, aneurysms, and cramps
HE-LFB: hematoxilin and eosin-luxol fast blue
HELLP: hemolysis, elevated liver enzymes, low platelets
ICH: intracerebral hemorrhage
ILAE: International League against Epilepsy
IVH: intraventricular hemorrage
IVF: in vitro fertilisation
IUGR: intrauterine growth restriction
L: left
MACS: manual ability classification system
MAP-2: microtubule-associated protein 2
MCD: malformation of cortical development
MEB: muscle-eye-brain
mMCD: mild malformation of cortical development
MRI: magnetic resonance imaging
mTOR: mammalian target of rapamycin
NC1: non-collagenous domain
NOS: not otherwise specified
P: percentile
Pat.: Patient
PET: positron-emission tomography

PVHI: periventricular hemorrhagic infarction
PVL: periventricular leukomalacia
R: right
SF: seizure freedom
SGA: small for gestational age
SPECT: single photon emission computed tomography
SWI: susceptibility weighted imaging
THX: triple helical domain
TORCH: Toxoplasma gondii, other viruses, Rubella, Cytomegaly virus and Herpes simplex
VM: ventriculomegaly
VUS: variant of unknown significance
WM: white matter
WMC: white matter change

8 Literature

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9 Attachments

9.1 A developmental and genetic classification for malformations of cortical development

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(I) MALFORMATIONS SECONDARY TO ABNORMAL NEURONAL AND GLIAL PROLIFERATION OR APOPTOSIS
- (A) SEVERE CONGENITAL MICROCEPHALY (MIC), pre-migrational reduced proliferation or excess apoptosis
  - (1) MIC with severe IUGR deficiency and short stature
    Clinically defined with AR inheritance
    - (a) Seckel syndrome with unknown cause (Shanske *et al*., 1997)
    - (b) MOPD syndromes with unknown cause
    - (c) Other MIC-IUGR syndromes
    - (d) Seckel syndrome with mutations in *ATR* at 3q22–q24 (O'Driscoll *et al*., 2003)
    - (e) MOPD type 2 with mutations in *PCNT* at 21q22.3 (Rauch *et al*., 2008)
    - (f) MOPD type 1 with mutations in *ORC1* at 1p32 (Bicknell *et al*., 2011)
    - (g) MOPD type 1 with mutations in *ORC4* at 2q22-q23 (Guernsey *et al*., 2011)
    - (h) MOPD type 1 with mutations in *ORC6* at 16q12 (Bernal and Venkitaraman, 2011)
    - (i) MOPD type 1 with mutations in *CDT1* at 16q24.3 (Bicknell *et al*., 2011*b*)
    - (j) MOPD type 1 with mutations in *CDC6* at 17q21.2 (Bicknell *et al*., 2011*a*)
  - (2) MIC with variable short stature (severe IUGR to mildly short), moderate to severe DD/ID, normal to thin cortex, SIMP, with/without callosal hypogenesis
    Genetically defined with AR inheritance
    - (a) Seckel syndrome or AR primary microcephaly (MCPH) with mutations in *CENPJ* at 13q12.12 (Al-Dosari *et al*., 2010)
    - (b) Seckel syndrome or MCPH with mutations in *CEP152* at 15q21.1 (Kalay *et al*., 2011)
  - (3) MIC with mildly short stature or normal growth, mild-moderate DD/ID, normal to thin cortex, with/without SIMP, with/without callosal hypogenesis and with/without focal PNH
    Clinically defined with AR inheritance
    - (a) AR primary microcephaly (MCPH) (Woods *et al*., 2005)
      Genetically defined with AR inheritance
    - (b) MCPH with mutations in *ASPM* at 1q31.3 (Bond *et al*., 2003; Shen *et al*., 2005; Desir *et al*., 2008)
    - (c) MCPH with mutations in *MCPH1* at 8p23.1 (Trimborn *et al*., 2004; Darvish *et al*., 2010)
    - (d) MCPH with mutations in *CDKRAP5* (Bond *et al*., 2005; W.B.D., in preparation)
    - (e) MCPH with mutations in *STIL* at 1p33 (Kumar *et al*., 2009)
  - (4) MIC with mildly short stature or normal growth, severe DD/ID, variable cortical development with SIMP or cortical dysgenesis and with/without ACC (includes genes with spectrum from SIMP to dysgenetic cortex or PMG)
    Clinically defined with AR or XL inheritance
    - (a) MIC with diffuse PMG
    - (b) MIC with asymmetric PMG
    - (c) MIC with atypical cortical dysgenesis
    Genetically defined with AR inheritance
    - (d) MCPH with mutations in *PNKP* at 19q13.33 (Shen *et al*., 2010)
    - (e) MCPH, MIC with diffuse PMG (MDP) or MIC with asymmetric PMG (MAP) with mutations in *WDR62* at 19q13.12 (Bilgüvar *et al*., 2010; Yu *et al*., 2010)
    - (f) MCPH, MDP (other cortical malformation) with mutations in *NDE1* at 16p13.11 (Alkuraya *et al*., 2011; Bakircioglu *et al*., 2011)
    - (g) MDP–MAP and ACC with mutations of *TBR2* (*EOMES*) at 3p24.1 (Baala *et al*., 2007)
  - (5) MIC with variable anomalies and less well characterized syndromes; with/without SIMP; with/without PNH, with/without CBLH
    Clinically defined with probable AR inheritance
    - (a) MIC with diffuse periventricular nodular heterotopia
    - (b) MIC with disproportionate cerebellar hypoplasia
    - (c) MIC (extreme) with jejunal atresia (Stromme *et al*., 1993)
    Genetically defined with AR inheritance
    - (d) MIC–PNH associated with mutations in *ARFGEF2* at 20q13.13 (Sheen *et al*., 2004; de Wit *et al*., 2009)
  - (6) MIC with severe DD/ID and evidence of degeneration, with/without mildly short stature, with/without enlarged extra-axial spaces, with/without ACC, with/without atypical cortical dysgenesis
    Clinically defined with AR inheritance
    - (a) MIC with enlarged extra-axial space
    - (b) MIC with enlarged extra-axial spaces and disproportionate cerebellar hypoplasia
    - (c) MIC due to foetal brain disruption with unknown cause

Genetically defined with AR inheritance
(d) Amish lethal microcephaly associated with mutations in *SLC25A19* at 17q25.1 (Rosenberg *et al*., 2002)
(e) MIC-capillary malformation syndrome (mutations in pending report)
(7) MIC with LIS (MLIS)—cortex thick or relatively thick, smooth white–grey border
Clinically defined with AR inheritance
(a) Barth MLIS syndrome
(b) Norman–Roberts MLIS syndrome
(c) MOPD1 variant with three-layer lissencephaly (Juric-Sekhar *et al*., 2011)
(d) MIC with lissencephaly, CBLH and Hirschsprung disease
(8) MIC with tissue loss and enlarged ventricles (hydrocephalus *ex vacuo* or hydranencephaly), with/without cortical dysplasia and with/without ACC
Clinically defined with presumed extrinsic (non-genetic) cause
(a) Foetal brain disruption sequence (Corona-Rivera *et al*., 2001)
Clinically defined with AR inheritance
(b) Familial foetal brain disruption-like syndrome with unknown cause
(c) Familial 'microhydranencephaly' with unknown cause (Behunova *et al*., 2010)
Genetically defined with AR inheritance
(d) Familial 'microhydranencephaly' associated with mutations of *MHAC* at 16p13.13–p12.2 (Kavaslar *et al*., 2000)

(B) MEGALENCEPHALY (MEG) including both congenital and early postnatal
(1) MEG with normal cortex (or presumably normal cortex)
Clinically defined with polygenic or AD inheritance
(a) Familial MEG
Genetically defined with AD inheritance
(b) Bannayan–Riley–Ruvalcaba syndrome, Cowden disease and MEG–autism with mutations in *PTEN* at 10q23.31 (Marsh *et al*., 1997; Marsh *et al*., 1999; Pilarski *et al*., 2011)
(c) Sotos syndrome with mutations in *NSD1* at 5q35.2–q35.3 (Türkmen *et al*., 2003)
(d) DD/ID, autism with *HEPACAM* mutations at 11q24.2 (AD, homozygous mutations cause AR megalencephaly with leukoencephalopathy and cysts) (López-Hernández *et al*., 2011)
(e) MEG, thumb anomalies and Weaver-like dysmorphism with dup 2p24.3 (includes *MYCN*)
Genetically defined with AR inheritance
(f) MACS syndrome with mutations in *RIN2* at 20p11.23 (Basel-Vanagaite *et al*., 2009)
Genetically defined with XL inheritance
(g) Simpson–Golabi–Behmel syndrome 1 with mutations in *GPC3* at Xq26.2 (Pilia *et al*., 1996)
(h) Simpson–Golabi–Behmel syndrome 2 with mutations in *OFD1* at Xp22.2 (Budny *et al*., 2006)
(i) MEG with DD/ID and seizures with mutations in *RAB39B* at Xq28 (Giannandrea *et al*., 2010)
Genetically defined with somatic mosaicism
(j) Proteus syndrome caused by somatic activating mutation in *AKT1* at 14q32.33 (Lindhurst *et al*., 2011)
(2) MEG with PNH—plus other anomalies
Clinically defined with AD or unknown inheritance
(a) MEG–PNH phenotype (Jan, 1999)
(3) MEG with PMG and other cortical dysgenesis
Clinically defined with unknown cause
(a) MCAP syndrome, includes MPPH (Mirzaa *et al*., 2004; Conway *et al*., 2007)
(b) Thanatophoric dysplasia or Apert syndrome with mutation of *FGFR3* at 4p16.3 (six-layered PMG-like cortex) (Hevner, 2005)

(C) CORTICAL DYSGENESIS WITH ABNORMAL CELL PROLIFERATION BUT WITHOUT NEOPLASIA
(1) Diffuse cortical dysgenesis
Genetically defined with AR inheritance
(a) PMSE syndrome with MEG, cortical dysgenesis including leptomeningeal glioneuronal heterotopia and cortical dysla-mination with mutations in *STRADA (LYK5)* (Puffenberger *et al*., 2007)
(2) Focal and multifocal cortical and subcortical dysgenesis
Clinically defined with putative postzygotic mosaicism
(a) HMEG isolated (Flores-Sarnat, 2002; Salamon *et al*., 2006; Mathern *et al*., 2007)
(b) HMEG with neurocutaneous syndromes (Flores-Sarnat, 2002)
(c) FCD Type II with large, dysmorphic neurons (FCDIIa) (Blümcke *et al*., 2011)
(d) FCD Type II with large, dysmorphic neurons and balloon cells (FCDIIb), including transmantle dysplasia and bottom of sulcus dysplasia (Blümcke *et al*., 2011)

Genetically defined with AD inheritance
(e) Tuberous sclerosis with cortical hamartomas and mutations of *TSC1* at 9q34.13 (Jones *et al.*, 1999; Crino *et al.*, 2006)
(f) Tuberous sclerosis with cortical hamartomas and mutations of *TSC2* at 16p13.3 (Jones *et al.*, 1999; Crino *et al.*, 2006)
(g) Tuberous sclerosis with HMEG (Galluzzi *et al.*, 2002)

(D) CORTICAL DYSPLASIAS WITH ABNORMAL CELL PROLIFERATION AND NEOPLASIA
(1) Neoplastic dysgenesis with primitive cells
(a) DNET
(2) Neoplastic dysgenesis with mature cells
(a) Ganglioglioma
(b) Gangliocytoma

(II) MALFORMATIONS DUE TO ABNORMAL NEURONAL MIGRATION
(A) MALFORMATIONS WITH NEUROEPENDYMAL ABNORMALITIES: PERIVENTRICULAR HETEROTOPIA
(1) Anterior predominate and diffuse PNH
Clinically defined with unknown cause
(a) Diffuse PNH with/without sparing of temporal horns
(b) Diffuse PNH composed of micronodules
(c) Diffuse PNH with frontonasal dysplasia (Guerrini and Dobyns, 1998)
(d) Anterior predominant PNH
(e) Anterior predominant PNH with fronto-perisylvian PMG (Wieck *et al.*, 2005)
(f) Unilateral or bilateral isolated PNH
Genetically defined with AD inheritance (new mutations)
(g) Anterior PNH with duplication 5p15.1 (Sheen *et al.*, 2003)
(h) Anterior or diffuse PNH with duplication 5p15.33 (Sheen *et al.*, 2003)
(i) Diffuse (but variable) PNH with del 6q27 (W.B.D, in preparation)
(j) PNH and Williams syndrome with del 7q11.23, including *HIP1* and *YWHAG* (Ferland *et al.*, 2006; Ramocki *et al.*, 2010)
(k) PNH with del 4p15 (gene not identified) (Gawlik-Kuklinska *et al.*, 2008)
(l) PNH with deletion 5q14.3–q15 (Cardoso *et al.*, 2009)
(m) PNH and agenesis of the corpus callosum with del 1p36.22-pter (Neal *et al.*, 2006)
Genetically defined with XL inheritance
(n) Bilateral PNH due to mutations of *FLNA*, with/without Ehlers–Danlos (Sheen *et al.*, 2001; Parrini *et al.*, 2006)
(o) PNH and Fragile X syndrome (Moro *et al.*, 2006)
(2) Posterior predominant (temporal-trigonal) PNH
Clinically defined with unknown cause
(a) Posterior PNH only
(b) Posterior PNH with hippocampal dysgenesis, colpocephaly, anomalies of midbrain tectum or cerebellar hypoplasia
(c) Posterior PNH with posterior PMG (Wieck *et al.*, 2005)
(3) Periventricular heterotopia, not nodular (unilateral or bilateral)
Clinically defined with unknown cause
(a) Diffuse PLH
(b) Frontal predominant PLH
(c) Posterior predominant PLH
(4) Ribbon-like heterotopia, bilateral undulating heterotopic band
Clinically defined with unknown cause
(a) Posterior predominant ribbon-like heterotopia
(b) Diffuse ribbon-like heterotopia

(B) MALFORMATIONS DUE TO GENERALIZED ABNORMAL TRANSMANTLE MIGRATION (radial and non-radial)
(1) Anterior predominant or diffuse classic (four-layered) LIS and SBH
Clinically defined with unknown cause
(a) Anterior predominant LIS with abrupt transition and cerebellar hypoplasia (previously LCHe)
(b) Anterior predominant or diffuse LIS (ILS)
Clinically defined with AR inheritance
(c) Anterior predominant LIS (ILS) with AR inheritance
(d) Winter–Tsukahara syndrome (Levin *et al.*, 1993)
Clinically defined with AD (new mutation) inheritance
(e) Baraitser–Winter syndrome with anterior or diffuse LIS–SBH (Rossi *et al.*, 2003)
(f) Anterior predominant LIS (ILS) or SBH with *DCX* mutation at Xq22.3–q23 (Dobyns *et al.*, 1999)

(2) Posterior predominant or diffuse classic (four-layered) and two-layered (without cell-sparse zone) LIS and SBH
Clinically defined with unknown cause
(a) Posterior predominant or diffuse LIS with brainstem and cerebellar hypoplasia, with/without ACC (includes former LCHa, LCHc, LCHd, LCHf (Ross *et al*., 2001))
(b) Posterior predominant or diffuse LIS (ILS) (Pilz *et al*., 1998, Dobyns *et al*., 1999)
(c) Diffuse LIS with hair and nail anomalies (Celentano *et al*., 2006)
(d) Perisylvian (central) pachygyria (ILS)
(e) Ribbon like deep white matter heterotopia with/without ACC, thin overlying cortex
Clinically defined with AD inheritance
(f) Posterior predominant SBH (Deconinck *et al*., 2003)
Genetically defined with AD inheritance (new mutation)
(g) Posterior or diffuse LIS with cerebellar hypoplasia or LIS (ILS) with *TUBA1A* mutations at 12q12-q14 (Poirier *et al*., 2007; Kumar *et al*., 2010)
(h) Miller-Dieker syndrome (four-layered) with deletion 17p13.3 (*YWHAE* to *LIS1*) (Dobyns *et al*., 1991)
(i) Posterior or diffuse LIS (ILS, four-layered) or posterior SBH with *LIS1* deletions or mutations at 17p13.3 (Dobyns *et al*., 1993; Pilz *et al*., 1999)

(3) X-linked lissencephaly (three-layered, without cell-sparse zone) with callosal agenesis, ambiguous genitalia (XLAG)
Clinically defined with unknown cause
(a) XLAG-like syndrome with temporal-posterior predominant LIS, ACC, microphthalmia and midline cleft lip and palate
(b) XLAG with temporal-posterior predominant LIS and ACC with mutations in *ARX* at Xp22.13 (Bonneau *et al*., 2002)

(4) Reelin-type LIS (inverted cortical lamination, without cell-sparse zone)
Clinically defined with AR inheritance
(a) Frontal predominant mild LIS with severe hippocampal and CBLH (Kato *et al*., 1999)
Genetically defined with AR inheritance
(b) Frontal predominant mild LIS with severe hippocampal and CBLH with *RELN* mutation at 7q22 (Hong *et al*., 2000)
(c) Frontal predominant mild LIS with severe hippocampal and CBLH with *VLDLR* mutation at 9p24 (Boycott *et al*., 2005)

(5) Variant LIS (other rare types exist but are poorly characterized)

(C) MALFORMATIONS PRESUMABLY DUE TO LOCALIZED ABNORMAL LATE RADIAL OR TANGENTIAL TRANSMANTLE MIGRATION

(1) Subcortical heterotopia (other than band heterotopia or cortical infolding), all clinically defined with unknown cause
(a) Curvilinear transmantle heterotopia, with thinning of overlying cortex, decreased volume of affected hemisphere, with/without ACC, with/without basal ganglia anomalies (Barkovich, 1996)
(b) Multinodular subcortical heterotopia with thin overlying cortex, with/without PMG (Barkovich, 2000)
(c) Transmantle columnar heterotopia with/without PNH

(2) Sublobar Dysplasia, clinically defined with unknown cause (Tuxhorn *et al*., 2009)

(D) MALFORMATIONS DUE TO ABNORMAL TERMINAL MIGRATION AND DEFECTS IN PIAL LIMITING MEMBRANE

(1) Dystroglycan–laminin complex abnormalities with cobblestone malformation complex (COB), with or without congenital muscular dystrophy
Clinically defined with AR inheritance but causative gene unknown
(a) Walker–Warburg syndrome (Dobyns *et al*., 1985, 1997)
(b) Muscle–eye–brain syndrome (Santavuori *et al*., 1989; Haltia *et al*., 1997)
(c) Congenital muscular dystrophy with CBLH (Italian MEB)
Genetically defined with frontal predominant COB and AR inheritance
(d) WWS or MEB with *POMT1* mutation at 9q34.1 (Beltran-Valero de Bernabe *et al*., 2002; van Reeuwijk *et al*., 2006)
(e) WWS or MEB with *POMT2* mutation at 14q24.3 (van Reeuwijk *et al*., 2005; Mercuri *et al*., 2006)
(f) MEB with *POMGnT1* mutation at 1p34–p33 (Manya *et al*., 2003)
(g) WWS, FCMD or FCMD with retinal abnormality (MEB-like) with *FKTN* mutation at 9q31 (Beltran-Valero de Bernabe *et al*., 2003, Manzini *et al*., 2008, Yoshioka, 2009, Yis *et al*., 2011)
(h) WWS or MEB with *FKRP* mutation at 19q13.3 (Beltran-Valero de Bernabe *et al*., 2004)
(i) WWS or MEB with *LARGE* mutation at 22q12.3-q13.1 (van Reeuwijk *et al*., 2007)
Genetically defined with posterior predominate COB and AR inheritance
(j) Posterior predominant COB and CMD with *LAMA1A* mutation at 18p11.31
(k) Posterior predominant COB with *LAMC3* mutation at 9q33–q34 (lacks CMD) (Barak *et al*., 2011)

(2) Cobblestone malformations in CDG
Genetically defined with AR inheritance
(a) CHIME-like syndrome with frontal predominant COB with *SRD5A3* mutation at 4q12 (Al-Gazali *et al*., 2008; Cantagrel *et al*., 2010)

(b) Debré-type cutis laxa with frontal predominant COB and *ATP6V0A2* mutation at 12q24.3 (Kornak *et al*., 2008; Van Maldergem *et al*., 2008)

(3) Cobblestone malformation with no known glycosylation defect

(a) Frontal predominant COB with *GPR56* mutations at 16q13 ('bilateral frontoparietal polymicrogyria') (Piao *et al*., 2002, 2005)

(b) Walker-Warburg syndrome secondary to *COL4A1* mutations at 13q34 (Labelle-Dumais *et al*., 2011)

(4) Other syndromes with cortical dysgenesis and marginal glioneuronal heterotopia, but with normal cell types

Clinically defined with extrinsic or unknown cause

(a) Foetal alcohol syndrome

Clinically defined with AR inheritance

(b) Galloway–Mowat syndrome

(III) MALFORMATIONS DUE TO ABNORMAL POSTMIGRATIONAL DEVELOPMENT

(A) MALFORMATIONS WITH PMG OR CORTICAL MALFORMATIONS RESEMBLING PMG

(1) PMG (classic) with transmantle clefts (schizencephaly) or calcification

Clinically defined with clefts suggesting vascular pathogenesis or unknown cause

(a) Schizencephaly (Barkovich and Kjos, 1992)

(b) Septo-optic dysplasia with schizencephaly (Barkovich *et al*., 1989)

Clinically defined with prenatal viral exposure (especially CMV)

(c) Schizencephaly with positive neonatal CMV testing (Iannetti *et al*., 1998)

(d) Diffuse or patchy PMG with periventricular calcifications and positive neonatal CMV testing

(e) Diffuse, patchy or perisylvian PMG with hearing loss and positive neonatal CMV testing

Clinically defined with AR inheritance

(f) Familial schizencephaly with single unilateral or bilateral clefts (Haverkamp *et al*., 1995)

(g) Familial schizencephaly with multiple bilateral clefts

(h) Band-like calcifications with PMG (pseudo-TORCH) (Briggs *et al*., 2008)

Genetically defined with AR inheritance

(i) Band-like calcifications with PMG (pseudo-TORCH) with mutations of *OCLN1* at 5q13.2 (O'Driscoll *et al*., 2010)

(2) Polymicrogyria without clefts or calcifications classified by location

Clinically defined bilateral PMG without clefts of unknown cause

(a) Generalized PMG (Chang *et al*., 2004)

(b) Frontal PMG (Guerrini *et al*., 2000)

(c) Perisylvian PMG (Kuzniecky *et al*., 1993)

(d) Posterior PMG (lateral parieto-occipital) (Barkovich *et al*., 1999)

(e) Parasagittal PMG

(f) Parasagittal mesial occipital PMG (Guerrini *et al*., 1997)

Clinically defined unilateral PMG without clefts of unknown cause

(g) Hemispheric PMG (Chang *et al*., 2006)

(h) Perisylvian PMG (Chang *et al*., 2006)

(i) Focal PMG (Barkovich, 2010a)

(3) Syndromes with PMG (neuropathology may differ from classic PMG)

Clinically defined syndromes with AD inheritance

(a) Adams–Oliver syndrome AD form (Snape *et al*., 2009)

Clinically defined syndromes with AR inheritance

(b) Adams–Oliver syndrome AR form (Snape *et al*., 2009)

(c) Joubert syndrome and related disorders with PMG, includes Meckel–Gruber, Arima (cerebro-oculo-renal) and Joubert syndromes with causative genes unknown (Gleeson *et al*., 2004)

Clinically defined syndromes with XL inheritance (probable)

(d) Aicardi syndrome (Aicardi, 2005)

(e) Oculocerebrocutaneous (Delleman) syndrome (Moog *et al*., 2005)

Genetically defined with AD inheritance (new mutations)

(f) Fronto-parietal PMG, variable ACC and delayed myelination of anterior limb internal capsule with *TUBB2B* mutations at 6p25.2 (Jaglin *et al*., 2009)

(g) Fronto-parietal PMG, variable with *TUBB3* mutations at 16q24.3 (Poirier *et al*., 2010)

(h) Knobloch syndrome with high myopia, vitreoretinal degeneration, retinal detachment, occipital cephalocele and variable PMG with *COL18A1* mutations at 21q22.3 (Sertié *et al*., 2000)

(i) Aniridia, variable temporal PMG, absent anterior commissure and pineal gland, and variable CBLH with *PAX6* mutations at 11p13 (Mitchell *et al*., 2003; Graziano *et al*., 2007)

(j) Perisylvian PMG with deletion 1p36.3 (gene not identified) (Dobyns *et al*., 2008)

(k) Perisylvian PMG with deletion 22q11.2 (gene not identified) (Cramer *et al*., 1996)

Genetically defined with AR inheritance

(l) Goldberg–Shprintzen (megacolon) syndrome with mutations of *KIAA1279* at 10q22.1 (Brooks *et al*., 2005)

(m) Joubert syndrome with variable (low penetrance) PMG and *AHI1* mutations at 6q23.3 (Dixon-Salazar *et al*., 2004; Valente *et al*., 2006)

(n) Meckel–Gruber syndrome with variable (low penetrance) PMG and *TMEM216* mutations at 11q12.2 (Valente *et al*., 2010)

(o) Generalized (versus perisylvian) PMG, ACC and optic nerve hypoplasia with *TUBA8* mutations at 22q11.21 (Abdollahi *et al*., 2009)

(p) Perisylvian PMG, ACC, delayed myelination of anterior limb internal capsule and cerebellar vermian hypoplasia with mutation of *TBR2* (*EOMES*) at 3p24.1 (Baala *et al*., 2007)

(q) Warburg Micro syndrome with mutations of *RAB3GAP1* at 2q21.3 (Morris-Rosendahl *et al*., 2010)

(r) Warburg Micro syndrome with mutations of *RAB3GAP2* at 1q41 (Borck *et al*., 2011)

(s) Warburg Micro syndrome with mutations of *RAB18* at 10p12.1 (Bem *et al*., 2011)

Genetically defined with XL inheritance

(t) Perisylvian PMG, rolandic seizures and speech-language dyspraxia with *SRPX2* at Xq22.1 mutations (Roll *et al*., 2006, 2010)

(u) Perisylvian PMG, mild MIC and thin body habitus with *NSDHL* mutation at Xq28 (McLarren *et al*., 2010)

(v) Perisylvian PMG with Xq27 locus (gene not identified) (Santos *et al*., 2008)

(w) Perisylvian PMG with Xq28 locus (gene not identified) (Villard *et al*., 2002)

(B) CORTICAL DYSGENESIS SECONDARY TO INBORN ERRORS OF METABOLISM (neuropathology differs from classic PMG)

Genetically and biochemically defined with AR inheritance

(1) Mitochondrial and pyruvate metabolic disorders

(a) Non-ketotic hyperglycinaemia with mutations of *GLDC* at 9p24.1, *GCSH* at 16q23.2 or *AMT* at 3p21.31

(b) Multiple Acyl-CoA dehydrogenase deficiency (Glutaric aciduria type II) with mutations of *ETFA* at 15q24.2-q24.3, *ETFB* at 19q13.41 or *ETFDH* at 4q32.1 (Govaert *et al*., 2004)

(2) Peroxisomal disorders

(a) Zellweger syndrome with mutation of many genes involved in peroxisomal biogenesis (Volpe and Adams, 1972; Steinberg *et al*., 2006)

(b) Neonatal adrenoleukodystrophy with mutation of many genes involved in peroxisomal biogenesis (Kamei *et al*., 1993)

(c) D-Bifunctional protein deficiency with *HSD17B4* mutation at 5q2 (Grønborg *et al*., 2010)

(C) FOCAL CORTICAL DYSPLASIAS (WITHOUT DYSMORPHIC NEURONS) DUE TO LATE DEVELOPMENTAL DISTURBANCES

Clinically/histologically defined and sporadic

(1) Minor malformations of Cortical Development (mMCD)

(2) Type I FCD

(a) Abnormal radial cortical lamination (Blümcke *et al*., 2011)

(b) Abnormal tangential cortical lamination (Blümcke *et al*., 2011)

(c) Abnormal radial and tangential lamination (Blümcke *et al*., 2011)

(3) Type III FCD

(a) Associated with hippocampal sclerosis (Blümcke *et al*., 2011)

(b) Associated with tumors (Blümcke *et al*., 2011)

(c) Associated with vascular malformations (Blümcke *et al*., 2011)

(d) Associated with other principal lesions during early life (Blümcke *et al*., 2011)

(D) POSTMIGRATIONAL DEVELOPMENTAL MICROCEPHALY (PREVIOUSLY POSTNATAL MIC) WITH BIRTH OFC –3 SD OR LARGER, LATER OFC BELOW –4 SD AND NO EVIDENCE OF BRAIN INJURY

(1) Postmigrational MIC with limited functional deficits

Clinically defined

(a) Postmigrational MIC with no cause or syndrome identified

Genetically defined with AD inheritance (sporadic new mutations)

(b) MIC and mild ID with *SHH* mutation (Ginocchio *et al*., 2008)

(c) MIC and variable ACC with deletion 1q43q44 (includes *AKT3*) (Hill *et al*., 2007)

(2) Postmigrational MIC with broad functional deficits consistent with a 'developmental encephalopathy' (Angelman-like, Rett-like class of disorders)

Clinically defined with AR inheritance

(a) PEHO syndrome (Salonen *et al*., 1991; Vanhatalo *et al*., 2002)

Genetically defined with AD inheritance (sporadic new mutations)

(b) Pitt–Hopkins syndrome with mutations of *TCF4* at 18q21.1 (Zweier *et al*., 2007)

(c) FOXG1 syndrome with deletions or mutations of *FOXG1* at 14q13 (Kortüm *et al*., 2011)

(d) Duplication of FOXG1 at 14q13 (Brunetti-Pierri *et al*., 2011)

Genetically defined with AD inheritance (or pathogenic *de novo* copy number variant) and imprinting effects

(e) Maternal duplication 15q11.2 (Kitsiou-Tzeli *et al*., 2010)

(f) Angelman syndrome with maternally deletion 15q11.2 or mutation of *UBE3A* at 15q11.2 (Matsuura *et al*., 1997)

Genetically defined with AR inheritance

(g) Pitt–Hopkins like syndrome with mutations of *NRXN1* at 2p16.3 (Zweier *et al*., 2009)

(h) Pitt–Hopkins-like syndrome with mutations of *CNTNAP2* at 7q35-q36 (Zweier *et al*., 2009)

(i) Pontocerebellar hypoplasia with mutations of *TSEN54* at 17q25.1, *TSEN2* at 3p25.1, *TSEN34* at 19q13.4, *RARS2* at 6q16.1 (Namavar *et al*., 2011)

Genetically defined with XL inheritance

(j) Rett syndrome with mutations of *MECP2* at Xq28 (Amir *et al*., 1999)

(k) Angelman-like syndrome with mutations of *SLC9A6* at Xq26.3 (Gilfillan *et al*., 2008)

(l) X-linked mental retardation and autistic features with mutations of *JARID1C* at xp11.22–p11.21 (Jensen *et al*., 2005; Abidi *et al*., 2008)

(m) X-linked MIC with disproportionate cerebellar hypoplasia with mutations of *CASK* at Xp11.4 (in females) (Najm *et al*., 2008)

From: (Barkovich et al. 2012), with permission. See arrows in page 4 and 5 for the classification of *COL4A1* mutations.

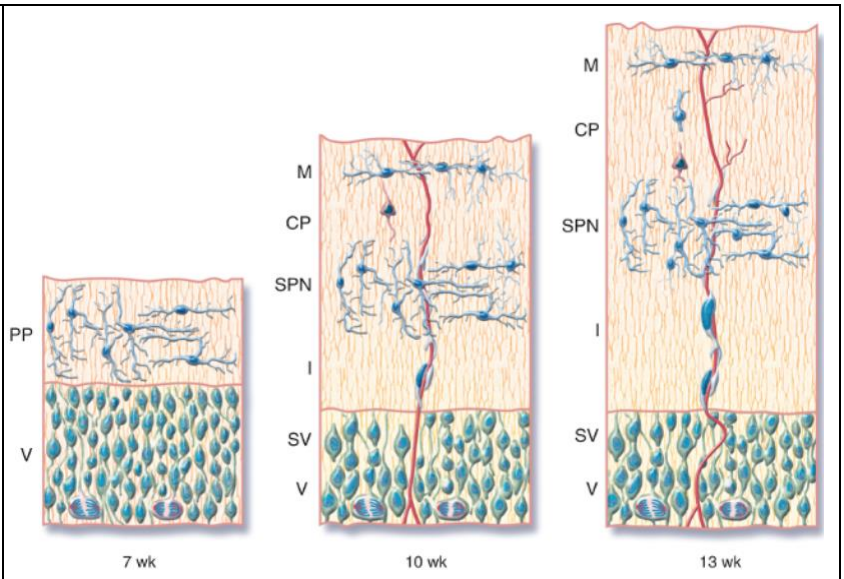
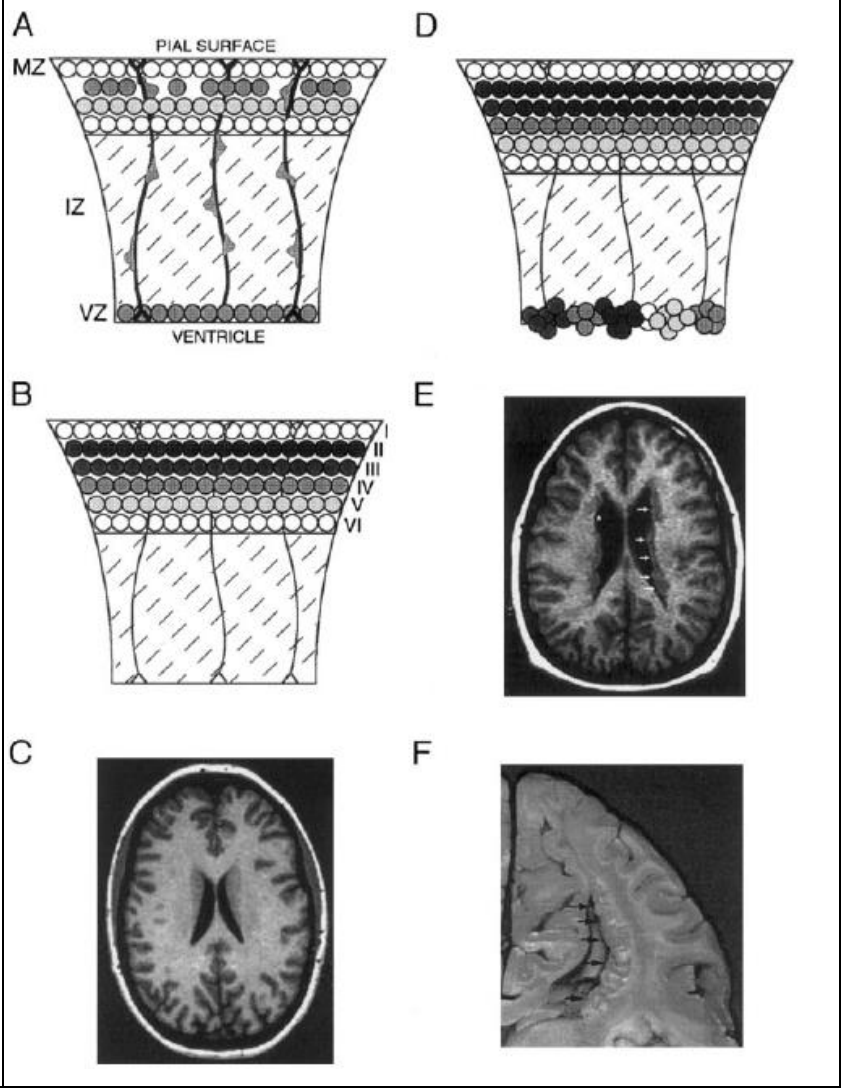
9.2 Collagen associated diseases

Gene	Disease	References, databases
COL1A1	Osteogenesis imperfecta	Marini et al. (2007)
COL1A2	Ehlers–Danlos syndrome	Dalgleish (1997, 1998) www.le.ac.uk/genetics/collagen Bodian and Klein (2009) http://collagen.stanford.edu/
COL2A1	Spondyloepiphyseal dysplasia Spondyloepimetaphyseal dysplasia, Achondrogenesis, hypochondrogenesis Kniest dysplasia, Stickler syndrome	Bodian and Klein (2009) http://collagen.stanford.edu/
COL3A1	Ehlers–Danlos syndrome	Dalgleish (1997, 1998) www.le.ac.uk/genetics/collagen Bodian and Klein (2009) http://collagen.stanford.edu/
COL4A1	Familial porencephaly Hereditary angiopathy with nephropathy, aneurysms and muscle cramps syndrome	Van Agtmael and Bruckner-Tuderman (2010)
COL4A3	Alport syndrome	Van Agtmael and Bruckner-Tuderman (2010)
COL4A4	Benign familial haematuria	
COL4A5	Alport syndrome	Bateman et al. (2009), Van Agtmael and Bruckner-Tuderman (2010)
COL4A6	Leiomyomatosis	
COL5A1	Ehlers–Danlos syndrome	Callewaert et al. (2008)
COL5A2		
COL6A1	Bethlem myopathy	Lampe and Bushby (2005)
COL6A2	Ullrich congenital muscular dystrophy	
COL6A3		
COL7A1	Dystrophic epidermolysis bullosa	Fine (2010)
COL8A2	Corneal endothelial dystrophies	Bateman et al. (2009)
COL9A1	Multiple epiphyseal dysplasia	Carter and Raggio (2009)
COL9A2		
COL9A3	Multiple epiphyseal dysplasia Autosomal recessive Stickler syndrome	Carter and Raggio (2009)
COL10A1	Schmid metaphyseal chondrodysplasia	Grant (2007)
COL11A1	Stickler syndrome Marshall syndrome	Carter and Raggio (2009)
COL11A2	Stickler syndrome Marshall syndrome Otospondylomegaepiphyseal dysplasia Deafness	Carter and Raggio (2009)
COL17A1	Junctional epidermolysis bullosa-other	Has and Kern (2010)
COL18A1	Knobloch syndrome	Nicolae and Olsen (2010)

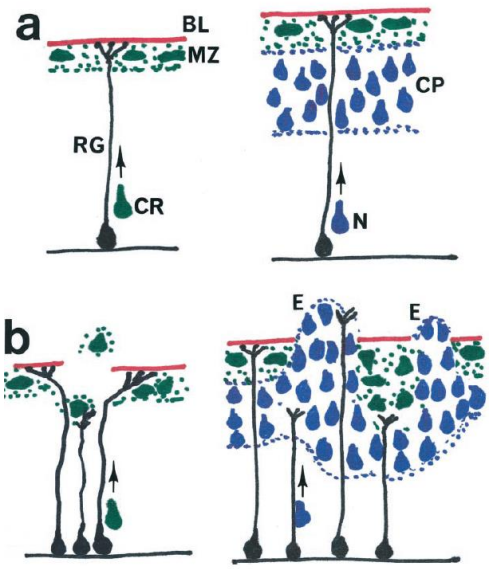
From: (Ricard-Blum 2011), with permission from editor "Cold Spring Harbour Perspectives on Medicine."

Other Collagen associated diseases:
Goodpasture Syndrome: Antibodies against α3chain of Collagen IV lead to kidney and lung involvement
Bronchiolitis obliterans is caused by strong immune response against Collagen V after lung transplantation
From: (Ricard-Blum 2011)

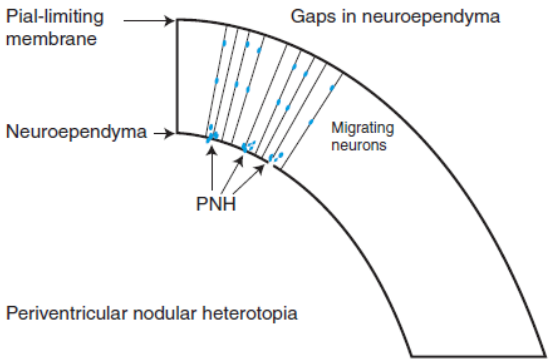
9.3 Neuronal migration and periventricular heterotopias

Structures involved in neuronal migration in human cortex, with permission from: (Volpe 2018, Ch.6). CP: cortical plate, I: intermediate zone, M: marginal zone, PP: preplate zone, SPN: subplate neurons, SV:subventricular zone, V:ventricular zone, wk: week. At the top: pial surface, Bottom: ventricular surface, red: radial glial fiber.	
During fetal brain development, neurons are generated in the germinal periventricular zone (VZ) and migrate in a tangential (interneurons) and radial (projection neurons) pattern (A). The peak time period in human fetal brain development is from the third to the fifth month of gestation (Volpe 2018, Ch. 6). Radial migration to the cortical plate is guided by radial glia cells. Early generated neurons take superficial positions, and late generated neurons a deep position in cortex (inside-out migration), then building the six cortical layers. Cajal-Retzius neurons migrate to the most superficial layer of the cortex (marginal zone, MZ) and release "Reelin", which is a stop sign leading to detachment of the neurons from the radial glial processes. After completion of migration, the radial glial cells condense to astrocytes losing their structure (Fox und Walsh 1999; Sidman und Rakic 1973). Neurons never starting neuronal migration are found in periventricular heterotopia (D, MRI and postmortem examination with PVH in E) C, normal MRI. (Figure A-F from: Fox und Walsh 1999, with permission from the editor).	

9.4 Models for understanding neuronal heterotopias related to BM impairment



Breaches in pial BM leading to neuronal heterotopias (Halfter et al. 2002), with permission from The Journal of Neuroscience.
Cajal-Retzius (CR) cells migrate along the radial glia to form the marginal zone (MZ), then stopping migration of cortical neurons (N) to form the cortical plate (CP). If breaches in the pial basal lamina (BL) are found migration of CR cells and of neurons is impaired, thus leading to ectopias (E).



Breaches in subependymal BM leading to periventricular heterotopias (Barkovich et al. 2015), with permission from Cold Spring Harbor Perspectives on Medicine.
Disruption of neuroependyma leads to impaired attachment of radial glia cells, and thus neurons are not able to migrate and remain as nodules near the germinative ventricular zone, leading to periventricular nodular heterotopia.

In the study of Halfter et al. 2002, BM impairment was caused by nidogen binding site mutation in laminin gene. This binding site is known to be needed for interaction with collagen IV network. Possible disruption of BM due to collagen IV as a main component of BM is conceivable and was shown in the study of Pöschl et al 2004.

Danksagung

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Eidestattliche Erklärung

Ich erkläre hiermit an Eides statt, dass ich die vorliegende Dissertation mit dem Thema „*Epilepsy in patients carrying COL4A1/COL4A2 mutations with focus on neuroimaging, epilepsy surgery and histology"* selbständig verfasst, mich außer der angegebenen keiner weiteren Hilfsmittel bedient und alle Erkenntnisse, die aus dem Schrifttum ganz oder annähernd übernommen sind, als solche kenntlich gemacht und nach ihrer Herkunft unter Bezeichnung der Fundstelle einzeln nachgewiesen habe.
Ich erkläre des Weiteren, dass die hier vorgelegte Dissertation nicht in gleicher oder in ähnlicher Form bei einer anderen Stelle zur Erlangung eines akademischen Grades eingereicht wurde.

Neusäß, 3. Juni 2021

Ort, Datum Ingrid Körber-Rosso

Veröffentlichungen

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- "Histopathology And Mri Findings In Two Children With *COL4A1/-2* Mutation Related Epilepsy " Koerber et al. ,13th European Congress on Epileptology Vienna, Austria, 26th - 30th August 2018, Volume 59, Issue S3, https://onlinelibrary.wiley.com/doi/epdf/10.1111/epi.14612
- "Neurologic phenotypes associated with *COL4A1/-2* mutations", Coautorenschaft, Zagaglia et al., Neurology 91 (22) 2018, e2078-e2088
- „Clinical course, epilepsy surgery and outcome in pediatric patients with *COL4A1/COL4A2* associated epilepsy", Postervorstellung im Rahmen der „Jahrestagung der Deutschen Gesellschaft für Kinder und Jugendmedizin 2019", Körber et al. Neuropediatrics 2019, 50(S 02): S1-S55, https://www.thieme-connect.com/products/ejournals/abstract/10.1055/s-0039-1698215