Molecular alterations in epilepsy-associated malformations of cortical development
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Citation for published version (APA):

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Download date: 11 Dec 2018
General introduction
Molecular alterations in epilepsy-associated malformations of cortical development
GENERAL INTRODUCTION

EPILEPSY

Epilepsy is a common neurological disorder which has already been described in the second millennium BC [1]. At that time there was little or no understanding of brain function and epilepsy was attributed to supernatural forces. The Greek physician Hippocrates (460-370 BC) was the first to reject those forces as cause of illness and early Greek medical writings in line with his teachings were collected in the Hippocratic corpus [2]. One of the writings, entitled ‘On the Sacred Disease’, contains the first description of epilepsy as a brain disorder with a natural cause. Nevertheless, in the following century’s epilepsy was still believed to be caused by supernatural forces and was used as one of the hallmarks to identify witches in the late Middle Ages. Even today, patients and their families still suffer from stigma and discrimination in many parts of the world [3].

Epilepsy affects approximately 1% of the human population worldwide, with an incidence of 40-70/100 000/year in the developed countries [3, 4]. Epilepsy is characterized by the recurrence of unprovoked seizures in which a seizure is defined by the International League Against Epilepsy (ILAE) as ‘a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain’ [5]. Seizures may lead to disturbance of sensation and emotion, consciousness impairment and uncontrolled repetitive movements dependent on seizure type [6, 7]. The several different seizure types can be divided into focal and generalized seizures, in which focal seizures involve a localized part of the brain and generalized seizures involve both hemispheres [5]. Epilepsy should not be considered as a single disorder, but rather as a group of syndromes, all involving abnormal electrical activity in the brain, with a variety of underlying causes. Epilepsy syndromes can be classified as idiopathic or symptomatic epilepsies [5]. Idiopathic epilepsies arise in the absence of structural abnormalities or an external cause and are in general thought to have a genetic origin. Symptomatic epilepsies have a known origin, which can be a structural brain lesion, such as a tumor or traumatic scar, an infection or febrile seizures during childhood. The underlying cause has not been determined in all syndromes and syndromes with no idiopathic origin and a presumed symptomatic origin were previously referred to as ‘cryptogenic’. However, the definition of cryptogenic is indistinct and should therefore be avoided [5]. As an alternative the term ‘probably symptomatic’ can be used.

In many patients diagnosed with epilepsy, seizures respond well to one or more of the available antiepileptic drugs (AEDs), nevertheless, a substantial proportion of patients (~30%) develop pharmacoresistant epilepsy despite optimized medical treatment [8]. This medically intractable form of epilepsy is more often associated with symptomatic epilepsies compared to other epilepsy syndromes [8] and drug refractoriness is a serious clinical problem which has devastating consequences on the patient’s quality of life. In a limited number of patients, focal brain lesions can be treated surgically to improve seizure control [9-11].
MALFORMATIONS OF CORTICAL DEVELOPMENT (MCDs)

Malformations of cortical development (MCDs) are a recognized cause of symptomatic intractable epilepsy in children and young adults. The growing understanding and use of neuroimaging, histological and genetic techniques over the past decades has revealed a large group of various MCDs [12]. This includes on one hand disorders affecting the cortical development of the whole brain accompanied with severe neurological problems and on the other hand mild forms of focal abnormalities which can be clinically silent. Barkovich and his colleagues has proposed and improved in following years a classification scheme which is based on the first stage of cortical development which is affected [13]. The different partly overlapping stages of the complicated development of the human cerebral cortex are: neural proliferation, migration and differentiation/cortical organization. Therefore the malformations are classified as proliferation, migration or organization disorders. Focal MCDs due to early defects in neural proliferation includes focal cortical dysplasia with balloon cells (FCD type IIb), tuberous sclerosis complex (TSC), hemimegalencephaly (HMEG) and glioneuronal tumors (ganglioglioma (GG) and dysembryoplastic neuroepithelial tumor (DNT)).

Histological characteristics of focal MCDs

Focal cortical dysplasia (FCD)

Focal cortical dysplasia (FCD) was first described by Taylor et al. in 1971 [14] and comprises a broad range of abnormalities in the cortical mantle with or without abnormal cell types such as cytomegalic (also called giant or hypertrophic) neurons, dysmorphic (synonymous with dysplastic) neurons and balloon cells [15, 16]. Focal cortical dysplasia (FCD) is most frequently encountered in pediatric epilepsy surgery programs [17] and according to the commonly used classification of Palmini [15] two types of FCD can be distinguished; type I and type II FCD. Type I FCD refers to architectural disturbances of the cortical lamination without (FCD type IA) or with (FCD type IB) cytoarchitectural abnormalities such as cytomegalic neurons (Table 1; [15]).

Table 1. Definitions of FCD with cytoarchitectural abnormalities

<table>
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<tr>
<th>Grade Palmini [15]</th>
<th>Characteristics</th>
<th>Definition of abnormal cell types</th>
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<tbody>
<tr>
<td>FCD type IA</td>
<td>Cortical dislamination</td>
<td>Cytomegalic (synonymous with giant or hypertrophic) neurons are abnormally large neurons with central nuclei, preserved pyramidal morphology, an apical dendrite and a high neuropilament content</td>
</tr>
<tr>
<td>FCD type IB</td>
<td>Cortical dislamination with cytomegalic neurons</td>
<td>Dysmorphic (synonymous with dysplastic) neurons are abnormal in morphology (with enlarged soma size) and orientation and have abnormal dendritic patterns and a high neuropilament content</td>
</tr>
<tr>
<td>FCD type IIA</td>
<td>Cortical dislamination with cytomegalic and dysmorphic neurons</td>
<td>Balloon cells are often huge in size with a thin cell membrane, pale eosinophilic cytoplasm and an eccentric locate nucleus (often more than one)</td>
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FCD type I can be represented as an isolated lesion, though mild re-organization of the cortical layers is often associated with other primary lesions including hippocampal sclerosis, tumors or vascular lesions. In these cases, FCD likely reflects the ongoing plasticity and/or response to injury of the maturing as well as adult cortex [18-20].

Type II FCD, also known as ‘Taylor-type’ FCD, is subdivided in type IIA and IIB, in which FCD type IIA (FCDIIA) is represented by loss of cortical lamination accompanied by cytomegalic neurons and dysmorphic neurons (Table 1). FCD type IIB (FCDIIB) is characterized by cortical dislamination (Fig. 1A) and the presence of cytomegalic neurons, dysmorphic neurons and balloon cells (Fig. 1B; Table 1). Balloon cells express immature cell markers as nestin and vimentin (insert b in Fig. 1B). In addition to the presence of abnormal cell types, increased proliferation of glial cells (gliosis) is a characteristic hallmark of FCD.

**Tuberous Sclerosis Complex (TSC)**

Tuberous sclerosis complex (TSC) is caused by germ line mutations in either the \textit{TSC1} or \textit{TSC2} gene [21, 22] and is characterized by the development of hamartomas in various organs including the heart, the kidney and the brain. Three major brain lesions have been identified in TSC: cortical tubers, subependymal nodules (SENs) and subependymal giant cell tumors (SGCTs, synonymous with subependymal giant cell astrocytomas (SEGA)) [23, 24]. At brain autopsy, cortical tubers can be recognized by the blurred boundary between the grey and white matter and they are firm to palpation. Cortical tubers are focal malformations which vary in size, may contain calcifications, and multiple tubers can be present in one individual. They are identified as high-signal lesions on magnetic resonance imaging (MRI) T2 sequences [24] and post-mortem MRI (Fig. 1C) combined with histological examination revealed areas of reduced myelin density (Fig. 1D). Microscopically, cortical tubers look similar to FCDIIB with disordered cortical lamination and the presence of abnormal cell types including dysmorphic neurons and giant cells, resembling the balloon cells in FCD type IIB (Fig. 1E). Similar to FCDIIB, gliosis is often observed in cortical tubers [23, 24]. Despite the neuropathological similarities between FCDIIB and TSC, these disorders are distinct entities as FCDIIB patients lack additional cerebral or extracerebral manifestations.

**Hemimegalencephaly (HMEG)**

Hemimegalencephaly is a rare and severe brain malformation characterized by diffuse and unilateral enlargement of one cerebral hemisphere (Fig. 1F; [25]). HMEG has been described as an isolated malformation or in association with various neurocutaneous syndromes, including the linear naevus sebaceous syndrome, TSC and proteus syndrome [25-27]. Increased cortical thickness accompanied by blurring of the cortex-white matter junction is characteristic for HMEG [28]. Similar to TSC and FCDIIB, the different cortical layers are disorganized and dysmorphic neurons and balloon cells are observed (Fig. 1G). In contrast to dysmorphic neurons, balloon cells are not specific for HMEG and they are present in less than half of the patients [28]. Excessive neuronal cells are observed in the molecular layer and in the white matter [27, 28].

**Ganglioglioma (GG)**

The developmental tumor ganglioglioma (GG) is the most frequently encountered tumor type in young patients with focal intractable epilepsy and is rare in adults [29]. GG are often located in the temporal lobe as well circumscribed lesions. Histologically, GG consists of a
mixture of dysplastic neurons and neoplastic glial cells (Fig. 2A-B). The dysplastic neurons lack uniform orientation and are abnormal in shape and size. The glial cells represent the proliferative cell population; however, the proliferation marker Ki67 is only expressed in about 1% of the cells [29, 30]. Another characteristic hallmark for GG is the expression of the stem cell epitope CD34 (Fig. 2C) in 70 to 80% of the patients in the neuronal component of the tumor, as a microglial or astroglial nature has been excluded for CD34 expressing cells [29, 31, 32]. CD34 is normally not expressed in the mature brain and expression of CD34 distinguishes GG from other low grade tumors such as the dysembryoplastic neuroepithelial tumor (DNT). Additional features frequently encountered in GG include calcifications and lymphoid infiltrates [29].

Dysembryoplastic neuroepithelial tumor (DNT)
Another glioneuronal tumor highly associated with intractable epilepsy is the dysembryoplastic neuroepithelial tumor (DNT). DNTs are histologically recognized by their complex nodular appearance (Fig. 2D), which might arise at the junction between the grey and white matter and, similar to GG, are frequently observed in the temporal lobe. The tumors are predominantly composed of oligodendroglial-like cells mixed with ‘floating’ neurons, the so-called simple variant of DNT (Fig. 2E; [33-35]). In the complex variant, glial nodules and/or foci of cortical dysplasia are associated with the characteristic glioneuronal element of DNT (Fig. 2F; [35]). The floating neurons present in DNT may show various cytological abnormalities, though they do not resemble dysplastic neurons as observed in other MCDs.
PATHOGENESIS OF FOCAL MCDs

Abnormal cell types in focal MCDs retain an immature phenotype

In normal human brain development, the majority of neural precursor cells are born in the ventricular (VZ) and subventricular (SVZ) zones and migrate radially to the cortical surface [36]. The earliest migrating cells form the preplate, which is split by the formation of the cortical plate by subsequent migrating cells into the marginal zone and the subplate.

Figure 3. Cortical development in humans
(A) Schematic representation of normal cortical development in humans at different gestational weeks (GW). (B) Hematoxylin/Eosin (HE) and Nissl stains. Explanation in text; VZ, ventricular zone; PP, preplate; MZ, marginal zone; CP, cortical plate; SP, subplate; IZ, intermediate zone; SVZ, subventricular zone; I to VI, six-layered cortex, WM, white matter.

Figure 2. Histological characteristics of GG and DNT
Panels A-C: GG. A: Hematoxylin/Eosin (HE) staining showing the mixture of dysplastic neurons lacking uniform orientation (arrows) and glial cells. B: GFAP immunoreactivity (IR) showing the neoplastic astroglial tumor component. C: CD34 IR in GG. Panels D-F: DNT: D: HE staining at low magnification showing the nodular appearance of DNT. E: HE staining showing ‘floating’ neurons between oligodendroglial-like cells. Insert in E: NeuN staining detects the neuronal component of the tumor. F. Neun staining showing adjacent cortical dislamination. Scale bar in F. A, B, E, F: 40 µm; C: 80 µm; D: 400 µm.
The marginal zone becomes the molecular layer (or layer I) and the subplate disappears several weeks before birth. The cortical neuronal layers II to VI develop from the cortical plate in an ‘inside-out’ mode, meaning that the first arriving neurons form the deeper layers (V/VI) and the later born neurons migrate through these neurons to form the superficial cortical layers (II/III) (Fig. 3; [37]). Radial migration is supported by radial glial cells which are, in addition to scaffolds for migration, identified as neural stem cells as they can differentiate into either neurons, astrocytes or oligodendrocytes [38, 39]. In addition to radial migration of projection neurons and interneurons born in the VZ of the dorsal forebrain, a subpopulation of interneurons are born in the ganglionic eminences (GE) of the ventral forebrain and migrate tangentially first, followed by radial migration to their final position [40].

The expression of CD34, CD133, MAP1B, nestin, vimentin, CRMP4, GFAPδ and DCX [41-48] in dysmorphic neurons and balloon cells or giant cells in either FCDIIB, TSC or HMEG support the hypothesis that these cells fail to mature properly. However, also more mature markers like GFAP, NeuN, MAP2, internexin and neurofilament proteins [42-44, 49] are observed, reflecting a heterogenous cell population. Filament proteins (vimentin, nestin, GFAPδ and neurofilaments) and microtubule associated proteins (MAPs) are critically involved in stable formation of the cytoskeleton and cell division, initiation of cell migration (i.e. attachment to radial glia) and the establishment of synaptic connectivity. Therefore aberrant expression of these proteins likely contributes to the observed cytological abnormalities and appropriate cortical lamination in these focal MCDs. The expression of immature markers is less extensively studied in the glioneuronal tumors (GG and DNT); nevertheless, neuronal expression of the stem cell marker nestin, normally not expressed in the mature brain, has been reported in both GG and DNT [50-52].

**Pi3k-mTOR signaling as pathological candidate pathway**

Of the MCDs classified as proliferation disorders with the presence of abnormal cell types described above, causative genes have only been identified for the tuberous sclerosis complex (TSC), namely TSC1 and TSC2 [21, 22]. Mutations in either the TSC1 or TSC2 gene are detected in over 80% of the individuals diagnosed with TSC [53]. The gene products, TSC1 (hamartin) and TSC2 (tuberin), act together by formation of heterodimers and antagonizes the phosphatidylinositol-3 kinase - mammalian target of rapamycin (Pi3K-mTOR) signaling pathway which regulates cell growth and proliferation (Fig. 4; reviewed in [54, 55]). Briefly, the TSC1-TSC2 complex can be inactivated either by phosphorylation of TSC2 by the critical mediator Akt [56] or by impaired protein function as a result of gene mutations in TSC1 or TSC2. TSC2 mediates the transition of active (GTP-bound) Rheb into inactive (GDP-bound) Rheb and loss of function of the TSC1-TSC2 complex results in activated Rheb and thereby the activation of mTOR [57]. Activation of mTOR increases cell size and proliferation by phosphorylation of the ribosomal protein S6 kinase (p70S6K) and the eukaryotic initiation factor 4E binding protein 1 (4E-BP1) (Fig. 4; [54, 55]). Overall, dysfunction of either TSC1 or TSC2 activates downstream mTOR signaling [58], which likely explains the abnormal enlargement of dysmorphic neurons and giant cells in cortical tubers.

Given the neuropathological similarities between focal MCDs, both TSC1 and TSC2 have been screened for mutations in FCDIIB and GG. Sequence alterations were observed in TSC1 in FCDIIB and TSC2 alterations in GG and in a distinct type of FCDIIB with mineralization,
however, true ‘loss of function’ mutations were not reported [59-61]. Nevertheless, activated mediators downstream of mTOR are expressed in dysmorphic neurons and balloon cells in FCDIIIB as well as in the dysplastic neuronal component of GG [52, 62, 63]. Activated mediators upstream of mTOR are also detected in FCDIIIB [64, 65], suggesting differences in the regulation of the signaling mechanisms. Interactions between components of the PI3K-mTOR pathway and ERM (ezrin/radixin/moesin) proteins [66], indicates that activation of PI3K-mTOR signaling might also be involved in the aberrant positioning of the dysplastic cells in FCDIIIB, TSC and GG [65-67].

**EPILEPTOGENESIS OF FOCAL MCDs**

Epileptogenesis is the complex process by which a normal brain develops epilepsy and comprises the period between the initial event (e.g. infection or trauma) and the first seizure. Several focal MCDs share neuropathological, molecular and clinical features, which suggest that they might also have common mechanisms of epileptogenesis. Initial studies on epileptogenesis focused comprehensively on neuronal properties, though astrocytes have been increasingly recognized as critical modulators during epileptogenesis [68, 69]. Astrocytes control the extracellular neurotransmitter and potassium levels, which may prevent excessive neuronal excitation [70, 71]. Dysmorphic neurons and balloon or giant cells were thought to be additional cell types critically involved in the epileptogenesis in focal MCDs. Nevertheless, electrophysiological studies suggested that balloon and/or giant cells are incapable to generate action potentials [72]. Dysmorphic neurons show signs of hyperexcitability and may contribute to the epileptogenesis [72, 73]. However, there is no evidence that dysmorphic neurons are the primary generators of epileptiform activity.
An important, but incompletely resolved, question is where the seizures associated with the focal MCDs originate. The circumscribed nature of the focal lesions might suggest that seizures originate in the lesions themselves, which is supported by the beneficial effects of surgical resection in the majority of patients [9, 74]. However, evidence suggests that the neuronal networks in the surrounding ‘perilesional’ zones or even throughout the brain distant from the lesion are involved in the initiation and particularly propagation of seizures [75-77], likely to be involved in the minority of patients who are not seizure free after surgical resection. Otherwise, low resolution of radiographic and electroencephalographic measurements leading to incomplete resection of the lesion may also account for the continuation of seizures. Nevertheless, several studies, including animal models of focal epilepsy, suggest that seizures may originate outside the focal malformations (reviewed in [78]). Therefore, extensive radiographic and electroencephalographic presurgical evaluation is necessary to improve seizure outcome after surgery.

The immune system and epileptogenesis

The brain has long been considered as an ‘immune privileged’ site as the blood brain barrier (BBB) limits the access of systemic immune cells into the brain. Nevertheless, inflammatory reactions do occur in the brain and are, in addition to infections, associated with various central nervous system (CNS) disorders including stroke, neurodegenerative (e.g. Alzheimer) and autoimmune (e.g. multiple sclerosis) disorders [79, 80]. Major players in inflammatory reactions in the brain are the resident cells, especially microglia and astrocytes. These cells produce inflammatory mediators when they are triggered by for example injured or dead cells, toxic CNS-proteins (e.g. β-amyloid) or bacteria or viruses [80]. Both beneficial (e.g. production of neurotrophic factors) and destructive (e.g. neuronal damage or death) effects have been reported for immune and inflammatory reactions in the CNS and the final effect depends on the microenvironment, exposure time and mediators produced [79, 81-83].

Increasing evidence obtained from various experimental models of epilepsy indicates that immune and inflammatory reactions are activated by seizures, demonstrated by the rapid production of inflammatory mediators like interleukin (IL)-1β, tumor necrosis factor (TNF)-α and IL-6 (reviewed in [82, 84, 85]). Of these cytokines, the effects and role in the CNS are most extensively studied for IL-1β. Seizure initiation and propagation is critically regulated by the excitatory neurotransmitters glutamate and its inhibitory counterpart GABA (see below) and IL-1β modulates the function of both glutamate and GABA receptors [86-90]. Excessive production of IL-1β after seizures is generally considered to increase the risk of excitotoxicity and neuronal excitability. The majority of additional studies in experimental models of seizures suggest that IL-1β is involved in seizure generation as pre-application of this cytokine induces and prolongs seizures [91-93], application of the natural occurring antagonist of the signaling receptor of IL-1β, IL-1Ra, has anticonvulsant effects [92, 94-96] and inactivation of caspase-1, an enzyme critically involved in the production of active IL-1β, results in a reduction of seizures and a delay of seizure onset [97].

In humans, the role of immune and inflammatory reactions in epileptogenesis is still under investigation. Increased cytokine expression levels in either serum or cerebral spinal fluid (CSF) in epileptic disorders have been reported [98-103], although stable cytokine levels have been reported as well [98, 104]. Increased expression of both mRNAs and proteins associated
with inflammation in brain tissue from patients surgically treated for drug-resistant epilepsies [105-107] provide more compelling evidence that immune and inflammatory reactions do occur in the human epileptic brain. Moreover, activated microglia (the major cytokine producers in the CNS) are prominently present in GG, DNT and FCDIIB, and the density of activated microglia correlates with both the duration of epilepsy and the seizure frequency in these patients [108, 109].

**GABA and glutamate receptors and epileptogenesis**

The main excitatory neurotransmitter in the CNS is the amino acid L-glutamate, while γ-aminobutyric acid (GABA) is known as the main inhibitory neurotransmitter. Glutamate acts via activation of either ionotropic receptors (iGluRs; ligand gated ion channels; NMDA, AMPA or kainate receptors) or metabotropic receptors (mGluRs; G-coupled receptors; mGluR1-8), while GABA activates either GABA\_A receptors (chloride transporters) or GABA\_B receptors (G-coupled receptors) [71, 110].

The expression and cellular distribution of several glutamate and GABA receptors subunits have been investigated in the previously mentioned focal MCDs. Lower mRNA expression levels of specific GABA\_A receptor subunits are detected in the dysmorphic neurons in FCDIIB [111], while, using both immunocytochemical and single-cell mRNA analysis, increased expression levels have been reported for several glutamate receptor subunits [111-113]. Comparable altered expression of both GABA and glutamate receptors, suggestive toward a more excitable state, has been reported in dysmorphic neurons and giant cells in cortical tubers of TSC and dysmorphic neurons in hemimegalencephaly [27, 114]. Increased expression of specific glutamate receptor subunits is also demonstrated in the neuronal component of both GG and DNT [52, 115]. Since the electrophysiological data suggest that balloon and/or giant cells are inexcitable, the functional consequence of the changed expression levels in these cells is questionable. Nevertheless, the changed expression in neurons most likely promotes hyperexcitability since the normal balance between inhibition and excitation will be disturbed.

Besides molecular alterations at the cellular level, disruption of the normal cortical organization and circuitry might critically contribute to the epileptogenesis as well. The electrophysiological balance between excitation and inhibition in the neuronal circuitry is critically controlled by GABAergic interneurons as they regulate the degree of glutamatergic excitation and impaired GABAergic function is associated with the development of epilepsy [116]. Disruption of GABAergic interneuron development results in impaired inhibitory control and has been implicated in the development of epilepsy and several behavior disorders, including autism [117]. An impaired GABAergic function is suggested in focal MCDs given that GABAergic interneurons are aberrantly organized or reduced in number in FCDIIB [118-120] and in TSC [121] and low numbers of inhibitory interneurons of different subtypes are present in GG and DNT [122, 123]. Changes in protein expression suggestive for a disturbed balance in excitatory and inhibitory transmission have also been observed in the perilesional cortex [123, 124]. Electrophysiological studies in FCD strengthen the hypothesis of an impaired GABAergic function by demonstrating physiological evidence of decreased GABA-mediated synaptic inhibition [125].
AIM AND OUTLINE OF THIS THESIS

Focal MCDs due to early defects in neural proliferation are a recognized cause of medically intractable epilepsy in children and young adults. The present treatment options include polytherapy with various antiepileptic drugs (AEDs) or surgical resection; however, seizure control is improved in a limited number of patients. To develop novel, more effective treatment strategies, a better understanding of the underlying molecular alterations involved in both the pathogenesis and epileptogenesis of these developmental disorders is needed. Therefore the major aim of this thesis is to identify genes, proteins or signaling pathways that are critically involved in the development of these focal MCDs and their epileptogenicity.

Chapter 2 deals with the questions whether the PI3K-mTOR signaling pathway is activated in the glioneuronal tumors (GG and DNT) and to what extent this expression pattern resembles other MCDs. Using serial analysis of gene expression (SAGE), the gene expression profile of focal cortical dysplasia (FCDIIA) have been studied (Supplement – Chapter 1) and one interesting gene higher expressed in FCDIIA is VEGFB. To further investigate the relevance of this finding, the expression of VEGF proteins and their signaling receptors is evaluated in FCDIIB. The suggested link between the immune system and epileptogenesis is explored in several focal MCDs, with the focus on IL-1β and related proteins in FCDIIB, GG and DNT in chapter 4. The expression of markers of both innate and adaptive immunity is studied in TSC in chapter 5. Genes related to the immune and inflammatory response are prominently higher expressed in GG and TSC specimens demonstrated with microarray analysis. An overview of the differentially expressed genes and processes identified with these microarray analyses is presented in chapter 6 for GG and in chapter 7 for TSC. The main inhibitory neurotransmitter GABA has an excitatory role during development which is controlled by cation-chloride cotransporters (CCTs, NKCC1 and KCC2). Deregulated expression of these CCTs was observed in GG (chapter 6) and their expression pattern in different MCDs, including FCDIIB, HMEG and GG, is presented in chapter 8. In order to further reveal possible epileptogenic mechanism in TSC, the expression pattern of metabotropic glutamate receptors is studied and described in chapter 9. Finally, the molecular alterations detected in the different focal MCDs are discussed in chapter 10.