Temporal lobe epilepsy from a translational perspective: roles of the glial potassium channel Kir4.1 and the water channel AQP4

In fulfilment of the requirement for the degree
Doctor of Philosophy

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2011

Series of dissertations submitted to the
Faculty of Medicine, University of Oslo
No. 1241

ISBN 978-82-8264-314-6

Cover: Inger Sandved Anfinsen.
Printed in Norway: AIT Oslo AS.

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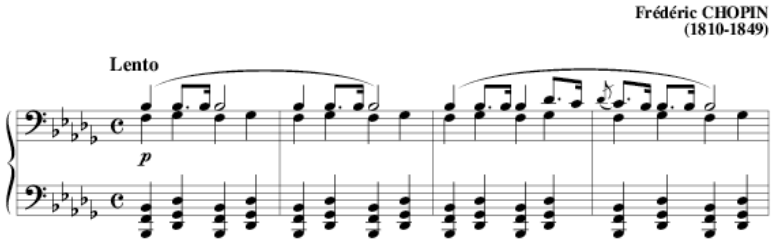
Preface

Frédéric Chopin is one of my favourite composers, as he combines romantic nostalgia with simplicity in the most ingenious manner. While I was working on my thesis, I immersed myself in several of his pieces and spent many hours in front of my piano, which is the best way for me to relax. During the work on my thesis I fell in love with the Piano Sonata No. 2 in B-flat minor, Op. 35, which he wrote in France during the years 1837-39. A part of this piece is shown here:



Chopin was plagued by bad health. Above all he had a pulmonary disease that probably caused his death at the age of 39. Chopin was a sensitive, reserved person with sudden attacks of melancholy, leading to the suggestion that he might have suffered from depression (Karenberg et al., 2007). Moreover, he suffered from hallucinations, as exemplified by an incident during a concert at a wealthy merchant's private house in Manchester on August 29th 1848. While he was playing the Sonata No. 2, he suddenly stood up and left the room. Later he described this incident in a letter with the words: "… I had played the Allegro and the Scherzo … and was about to play the March (notes above) when, suddenly, I saw emerging from the half-open case of my piano those cursed creatures…" (Caruncho and Fernández, 2010). Episodes like these were not uncommon for Chopin, but he preferred to keep them to himself.

As if specifically intended for this thesis, only shortly before submission of my work, a paper entitled "The hallucinations of Frédéric Chopin" was published in the journal "Medical Humanities" (Caruncho and Fernández, 2010). In this paper it is postulated for the first time that Chopin might have suffered from Temporal Lobe Epilepsy (TLE) – the condition of central interest to this thesis. Chopin's complex hallucinatory episodes are reviewed in the article and found to be consistent with seizures typical for TLE. Indeed, the attacks described in the paper could be focal seizures, attended

by complex visual experiences and/or psychic symptoms. TLE is frequently accompanied by anxiety, fear, insomnia, and depression, all symptoms that characterised the personality of Chopin.

Chopin once said “…Time is the best censor, and patience a most excellent teacher”, a sentence that also can be extrapolated to this thesis, and, indeed, to other important issues in life.

Acknowledgements

This thesis is based on collaboration between the Department of Neurology at Rikshospitalet, Oslo University Hospital (OUS), The Centre for Molecular Biology and Neuroscience (CMBN), a Norwegian Centre of Excellence at the University of Oslo, and Centre for Molecular Medicine Norway, the Nordic EMBL Partnership. It was carried out in parallel with my clinical work during the years 2007 to 2011, financed by the Department of Neurology at OUS, Rikshospitalet. A part of this thesis was conducted at the Departments of Laboratory Medicine and Neurosurgery at Yale University School of Medicine, New Haven CT, USA, funded by NNF, the Norwegian Association of Neurologists, and NES, the Norwegian Chapter of the International League against Epilepsy (ILAE).

First and foremost I want to thank all the patients with epilepsy
who used their ink and blood making this thesis possible

I wish to express my deepest respect and gratitude to my supervisor Professor Erik Taubøll for his formidable personal and professional guidance. Through the process of this research, he became a father figure to me. He inspired me with his great knowledge and enthusiasm and gave me space in which to develop myself.
I am totally indebted to my co-supervisor, Senior Scientist Erlend A. Nagelhus, for his steady support and solution-driven and intelligent guidance.
I sincerely appreciate the support from two great personalities in Norwegian and International Research, Professor Leif Gjerstad, and Professor and President of the University of Oslo, Ole Petter Ottersen. Without their support this work would not have been realizable.
Professor Nihal de Lanerolle and Associate Professor Tore Eid supervised my work at Yale University and spent their valuable time on an obstinate foreigner. Thanks for the support and patience. Heartfelt thanks also to Ilona Kovacs, one of the strongest and most amazing people I have met in an extraordinary period of her life.
I owe many thanks also to the Head of the Department of Neurology at OUS, Rikshospitalet, Professor Espen Dietrichs, for his consistent and generous background support.

To all the other fellows and co-authors that assisted in bringing this thesis to fruition and contributed with constructiveness and merriness, especially Fredrik, Eystein and Ulf, I am eternally grateful. Many thanks also to Milada for her significant contribution.
Warm and heartfelt thanks to my parents, who paved the way for me to reach my goals in life.
Last, but not least, I want to thank my wife, Elisabeth, for devoting immeasurable patience to me, and my children, Rebecca Sofie and Sebastian, who teach me every day that imagination is truly more important than facts.

Abbreviations

ADAM 22/ 23	A disintegrin and metalloproteinase domain 22/ 23
ADPEAF	Autosomal-dominant partial epilepsy with auditory features
AED	Antiepileptic drug
AQP	Aquaporin
AQP4	Aquaporin-4
ATP	Adenosine triphosphate
BBB	Blood-brain barrier
CI	Confidence interval
CNS	Central nerve system
CNV	Copy number variation(s)
CPS	Complex partial seizures
CSF	Cerebrospinal fluid
DAPC	Dystrophin-associated protein complex
EAST	Epilepsy, ataxia, sensorineural deafness and renal tubulopathy
ECM	Extracellular matrix
EEG	Electroencephalogram
EGFP	Enhanced green fluorescent protein
ECS	Extracellular space
FEB 1-9	Gene loci for febrile convulsion genes 1-9
FLAIR	Fluid attenuation inversion recovery
FS	Febrile seizure(s)
FLTLE	Familial lateral temporal lobe epilepsy
FMTLE	Familial mesial temporal lobe epilepsy
FTLE	Familial temporal lobe epilepsy
GEFS+	Generalized epilepsy with febrile seizures plus
GFP	Green fluorescent protein
GluR	Glutamate receptor
GluT	Glutamate transporter
GWAS	Genome-wide association study
hGFAP	Human glial fibrillary acidic protein
HHV6	Human herpes virus 6
HS	Hippocampal sclerosis
ICF	Informed consent form(s)
ILAE	International League against Epilepsy
IPI	Initial precipitating incident

Kir	Inwardly rectifying potassium channel
KCNJ10	Gene encoding Kir4.1
Kir4.1	Inwardly rectifying potassium channel subtype 4.1
KO	Knockout
LDA	Linear discriminant analysis
LTLE	Lateral temporal lobe epilepsy
MaMLTE	Mesial temporal lobe epilepsy with mass lesion
MMP	Matrix metalloproteinase
MTLE	Mesial temporal lobe epilepsy
MTLE-HS	Mesial temporal lobe epilepsy with hippocampal sclerosis
MRI	Magnetic resonance imaging
NIH	National Institutes of Health
NTLE	Neocortical temporal lobe epilepsy
OR	Odds ratio
PB	Phosphate buffer
PCR	Polymerase chain reaction
PDZ	Acronym combining the first letters of three proteins: post synaptic density protein, Drosophila disc large suppressor, and zonula occludens-1 protein. Common structural domain in signalling proteins
PLS	Partial least squares
PMTLE	Pseudo mesial temporal lobe epilepsy
REC	Regional ethics committee
SCN1/1A/2	Sodium channel, voltage gated, type I/ I alpha subunit/ II gene locus
SE	Status epilepticus
SeSAME	Seizures, sensineural deafness, ataxia, mental retardation and electrolyte imbalance
SMEI	Severe myoclonic epilepsy in infancy
SNP	Single nucleotide polymorphism
TLE	Temporal lobe epilepsy
TTX	Tetrodoxin
UCLA	University of California, Los Angeles
UCSC	University of California, Santa Cruz
WHO	World Health Organization

Translational research

"Progress is often made at the interface of disciplines and in an environment of intellectual freedom" (Zerhouni et al., 2005). The idea of translational research embraces this philosophy. In the narrow sense, translational research can be considered as a way of thinking and conducting scientific research with the aim of translating new knowledge, mechanisms, and techniques that have been generated by basic science research into novel approaches for prevention, diagnosis, and treatment of disease, or, in reverse, testing hypotheses generated by clinical observation in the lab (Marincola et al., 2003).

Translational research has become a centrepiece of Research Councils around the world. Governmental agencies as, for example, the National Institutes of Health (NIH) in the United States and the European Commissions, consider translational research a funding priority and annually increase their budgets for health-related translational programmes (Zerhouni et al., 2003). Academic centres, foundations, hospitals, and industry all establish translational research agendas, and several journals (e.g. *Translational Medicine* and *The Journal of Translational Medicine*) are committed to the subject. Inevitably the question arises: "what this is all about?" and "why is the translational approach promising?" The answer is simple. The rate of translation of basic science promises into clinical applications over the past decades has been disappointing and inefficient (Contopoulos-Ioannidis et al., 2003). In addition, it has been confirmed that a large quantity of clinical research resulted in the generation of information that was incorrect, obsolete, or even hazardous to human health (Antman et al. 1992). A bidirectional flow of information and multidisciplinary competence should be a powerful tool to address these inadequacies and one that is necessary in order to progress in our understanding of biological systems (Ioannidis et al. 2004). In the light of a growing awareness of the extreme complexity in the patterns of aetiology and pathogenesis in common diseases, the translational research approach seems, at least, to have a chance of providing benefit to human health and wellbeing.

Objectives of this thesis

Epilepsy comprises a range of different chronic brain disorders and syndromes characterised by spontaneous, recurrent, unprovoked seizures. Worldwide, epilepsy affects 50 million people, or between 0.7 and 1 % of the global population, occurring in all ages and in all socioeconomic groups (Zarrelli et al., 1999). According to the WHO, epilepsy accounts for 1 % of the global burden of disease, equivalent to breast cancer in women or lung cancer in men (http://www.who.int).

Aside from the impairment caused by the seizures themselves, epilepsy also interferes to a considerable extent with the normal activities of daily living, employment status, reproductive function, and social relationships, and is a significant risk factor for depression, serious injury, and death.

While 2/3 of all epilepsy patients respond successfully to currently available antiepileptic drugs (AEDs), 1/3 continues to have recurrent seizures despite optimised pharmacological treatment (Kwan and Brodie, 2000). These pharmacoresistant cases are mainly represented by patients suffering from Temporal Lobe Epilepsy (TLE), traditionally defined by seizure origin in, or involvement of, the temporal lobe. These patients should derive benefit from new therapies that will produce greater efficacy than current medications. As a prerequisite to fulfilling the unmet medical needs of patients with TLE, a clear delineation of the phenotypes, genotypes, and molecular pathways that underlie the generation or development of epileptic seizures is required.

Current antiepileptic treatment concepts are mainly based on the principle that epileptic seizures arise from abnormal excessive or synchronous *neuronal* activity in the brain. Undeniably, epileptic seizures could not occur without *neurons*. However, evidence has accumulated over recent years that proper neuronal functioning is not possible without the most abundant cell type in the brain, the *glial cells*. Glial cells, and especially astrocytes, are critically involved in maintenance of ion and water homeostasis, and hence in the pathophysiological mechanisms that lead to neuronal hyperexcitability and epilepsy (Seiffert et al., 2006). This makes glial cells an exciting subject for novel treatment concepts in epilepsy, especially in pharmacoresistant TLE.

Investigation of the molecular function of glial targets, and searching for genetic associations in hypothetical candidate genes, will assist in our understanding of the

pathogenic mechanisms in epilepsy, and specifically in subgroups of TLE. To contribute to this search is the primary goal of this thesis.

Detection of novel therapeutic targets for TLE is best performed by a translational research approach, as applied in this thesis, including:

1) *Phenotype studies* that aim to filter out clinically distinct subgroups on the basis of demographic data and common phenotypic features from a principally unselected TLE population. This facilitates genetic and biomedical research, which, in turn, provides a basis for tailored pharmacological treatment and improved outcome.
2) *Genetic association studies* in TLE subpopulations on candidate genes based on plausible *a priori* hypotheses, derived from biomedical or clinical/epidemiological research.
3) *Biomedical research* on glial/astrocyte targets in the TLE field, based on novel general concepts and on indications derived from own genetic and clinical studies.

List of papers

I. Heuser K, Taubøll E, Nagelhus EA, Cvancarova M, Ottersen OP, Gjerstad L. Phenotypic characteristics of temporal lobe epilepsy: the impact of hippocampal sclerosis. Acta Neurol Scand Suppl. 2009;(189):8-13

II. Heuser K, Nagelhus EA, Taubøll E, Indahl U, Berg PR, Lien S, Nakken S, Gjerstad L, Ottersen OP. Variants of the genes encoding AQP4 and Kir4.1 are associated with subgroups of patients with temporal lobe epilepsy. Epilepsy Res. 2010 Jan;88(1):55-64

III. Heuser K, Hoddevik EH, Taubøll E, Gjerstad L, Indahl U, Kaczmarek L, Berg PR, Lien S, Nagelhus EA, Ottersen OP. Temporal lobe epilepsy and matrix metalloproteinase-9: a tempting relation but negative genetic association. Seizure. 2010 Jul;19(6):335-8

IV. Heuser K, Cvancarova M, Gjerstad L, Taubøll E. Is Temporal Lobe Epilepsy with childhood febrile seizures a distinctive entity? A comparative study. Seizure. 2011 Mar;20(2):163-6

V. Heuser K, Eid T, Lauritzen F, Thoren AE, Vindedal GF, Taubøll E, Gjerstad L, Spencer DD, Ottersen OP, Nagelhus EA, de Lanerolle NC. Loss of Kir4.1 potassium channels in hippocampus of patients with mesial temporal lobe epilepsy.
Manuscript submitted

1. Introduction

1.1 The clinical picture of Temporal Lobe Epilepsy

1.1.1 Definition and classification

The classification of Temporal Lobe Epilepsy (TLE) has been frequently refined over the years, and the latest version is most probably only temporary also. These intermittent modifications have caused, and still cause, confusion. In the ILAE classification of 1985, TLE was defined as recurrent unprovoked seizures with origin in the temporal lobe (Proposal for classification of epilepsies and epileptic syndromes, 1985) With this definition, TLE was characterised as one of 5 symptomatic, localisation-related epilepsies, in which seizure semiology and epileptic activity in EEG define a link to a circumscribed anatomical region of the brain. According to the location where seizures arise, TLE was further divided into lateral (or neocortical) temporal lobe epilepsy (LTLE or NTLE) and medial/mesial temporal lobe epilepsy (MTLE). One principal pitfall is embodied in this anatomical classification, as the site of actual seizure origin may be at considerable distance from the region that gives rise to clinical symptoms (Lee et al., 2000). Hence, the revised classification of 1989 moved away from the anatomical classification and defined TLE on the basis of typical clinical features, and EEG and imaging findings (Proposal for revised classification of epilepsies and epileptic syndromes, 1989). However, the 1989 classification did not consider the aetiology or pathogenesis of TLE. TLE, in fact, denotes a variety of conditions with different aetiological backgrounds, most of which still are elusive.

The latest classification, published just last year, omits TLE as a separate category (Berg et al.2010), and instead highlights only *familial temporal lobe epilepsies* as a definite subgroup among electroclinical syndromes of adolescents/adults. In addition, *mesial temporal lobe epilepsy with hippocampal sclerosis (MTLE-HS)* is included as a distinctive constellation.

The latest classification also encompasses a number of conceptual modifications. According to current knowledge, all epileptic seizures involve neuronal networks in one or both hemispheres, engaging both cortical and subcortical structures. On the basis of this concept, the terms “focal” and “generalised” seizures have been

redefined. Thus, until further re-classification “focal” indicates that seizures primarily arise in one cerebral hemisphere, either in circumscribed areas, or with a wider distribution, while the term “generalised” refers to seizure generation in bilateral networks. Furthermore, the distinction between “simple partial” and “complex partial” seizures has been discarded, and the term “partial” is now to be used synonymously with “focal”.

These latest modifications may lead to confusion, including with respect to this thesis. For practical reasons, both definitions are used in the text of this thesis.

1.1.2 Historical notes

The English neurologist John Hughlings Jackson (1835-1911) is usually credited with being the first to recognise TLE. Through his description of “uncinate group of fits” and the “dreamy state” (over-consciousness or heightened intellectual state) in 1880, Jackson presented the first characterisation of TLE with medial seizure origin (Jackson, 1880; Jackson 1898; Hogan RE et al., 2003). Parallel to the work of Jackson, the German neuropsychiatrist and pathologist Wilhelm Sommer (1852-1900) reported evidence for a causal relationship between epilepsy and lesions in the hippocampus in an extensive autopsy material (Sommer, 1880). Sommer’s studies were extended by detailed histological observations by the German psychiatrist and neurologist Emil Bratz (1867-1934), who was the first to describe the characteristic morphological changes in hippocampal sclerosis (HS) (Bratz, 1899). The invention of EEG in the early 20th century contributed to increasing further our recognition and understanding of TLE. In 1937, the American neurologist Frederic A. Gibbs (1903-1992) introduced the term “psychomotor attacks” to describe the signs and symptoms of seizures with temporal lobe origin, and contributed to the general understanding of epilepsy as a state based on paroxysmal cerebral dysrhythmia (Gibbs et al., 1937). By the middle of the 20th century, TLE was widely established as a distinctive epileptic condition, partly due to the observation of focal temporal sharp waves in EEG of patients with clinically characteristic seizures (Jasper and Kershman, 1941). The first operations on patients with TLE were performed by Gibbs in 1951 and, shortly afterwards, by Falconer in the beginning of the fifties (Bailey and Gibbs, 1951; Falconer, 1953). As tissue from operated patients was available for investigation, extensive knowledge on pathological features in the epileptic hippocampus could be obtained. However, despite intensive research, the distinct role of the hippocampus

in the generation of seizures and the development of epilepsy remains unresolved, even today.

1.1.3 Epidemiology

It is often reported that 40 % of epilepsy patients experience complex partial seizures (CPS) (according to the previous classification), which are usually associated with seizure origin in the temporal lobe (Engel J Jr, et al., 2003). Naturally, this definition has limitations, as not all patients with CPS suffer from TLE. Moreover, it is often stated that one third of all epilepsy patients have TLE, but there is no conclusive evidence that this is correct.

A prerequisite of all epidemiological studies is clear definition of the population of interest. However, as described, TLE comprises a large variety of different entities with different aetiological backgrounds, most of which are still elusive. Another confusing factor is the frequent changes in definitions and classifications. This undermines epidemiological approaches, and means that there are no definitive epidemiological data on TLE. As MTLE-HS has recently been described as a distinctive entity (Wieser et al., 2004) appropriate epidemiological studies on this syndrome should now be possible. However, data about MTLE-HS are usually derived from large centres and generally focus upon surgically-treated patients and/or the most medically intractable cases. Information on mild forms of MTLE-HS is difficult to obtain, complicating the assembly of valid epidemiological data.

1.1.4 Anatomy of the hippocampus

The hippocampus plays an important role in the consolidation of information from short-term memory to long-term memory, and in spatial navigation. The hippocampal formation is located in the basomedial part of the temporal lobe of the brain and constitutes a major part of the archicortex and the limbic system. A cross section perpendicular to the long axis of the structure reveals the internal structure as two interlocking “C” shapes, one of which comprises the cornu ammonis (CA), the other makes up the dentate gyrus. The CA can be further segregated according to the anatomical classification by Lorente de No, (1934), in subregions CA1 – CA4. The CA is comprised of several layers as illustrated in figure 1.

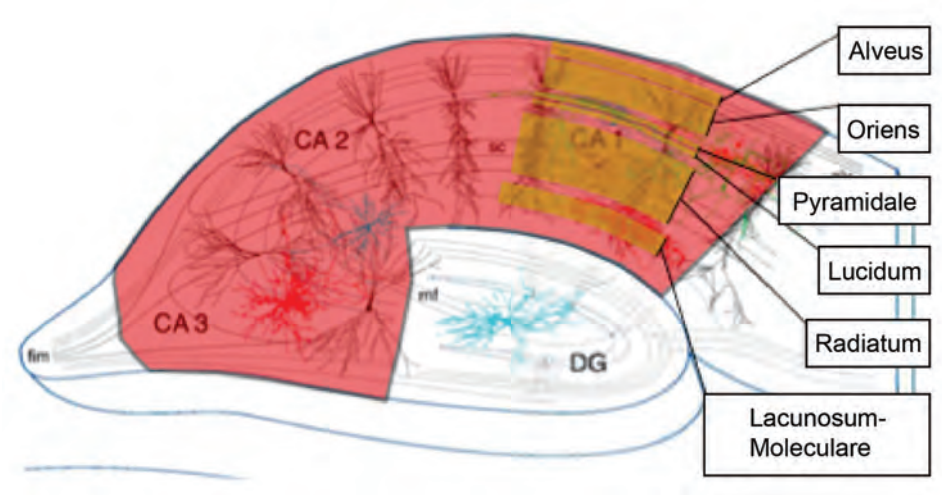


Figure 1: Main layers in the hippocampus proper

Alveus: most superficial layer and contains commissural fibers of pyramidal cells via the fimbria.

Stratum Oriens: contains inhibitory basket cells and the basal dendrites of pyramidal neurons (innervated by recurrent collaterals and contralateral hippocampus).

Stratum Pyramidale: contains the somas of pyramidal neurons. Mossy fibers (MFs) also synapse in this layer.

Stratum Lucidum: mossy fibres transverse and terminate in this region.

Stratum Radiatum: contains the Schaffer collaterals of CA3-1 pathway.

Stratum Lacunosum-Moleculare: also contains some fibers from the Schaffer collaterals.

The hippocampus has plenty of connections to and from surrounding brain regions. Afferent pathways to the hippocampus have their origin in the posterior parietal association cortex (area 7) and from the adjacent temporal and occipital regions (areas 22, 39, and 40). Most afferences penetrate the hippocampus via the entorhinal cortex. Moreover, direct afferences from amygdala also exist, from the hypothalamus, the septum region, and from the contralateral hippocampus via fornix.

The efferences of the hippocampus exit via alveus and fimbria towards the same regions from which the afferences originate.

Pathological changes of the hippocampus are typical for the syndrome of MTLE-HS, which is described in the following chapter.

Large textbooks have been written about the anatomy of the hippocampus. To detail the anatomical structure and integration of the hippocampus is not intended at this point. A comprehensive survey about hippocampal anatomy can, for example, be found in Per Andersen´s textbook entitled Hippocampus (Andersen, ed. 2007).

1.1.5 Mesial Temporal Lobe Epilepsy with hippocampal sclerosis

The hippocampus is one of the most studied anatomical regions of the brain, and hippocampal sclerosis (HS or Ammon´s horn sclerosis) the most explored "epileptogenic lesion". It is therefore not possible to review here all aspects of the extensive literature in this field. Over the years, evidence has accumulated that MTLE-HS may constitute a distinctive syndrome. In 2004, a panel consisting of leading experts discussed the definition, natural history, pathological features, pathogenesis, electroclinical, neurophysiological, neuropsychological, structural, and functional imaging features, as well as surgical outcomes in patients with HS and mesial seizure origin (MTLE-HS) (Wieser et al., 2004). This resulted in a consensus, in which MTLE-HS was considered to represent "a sufficient cluster of signs and symptoms to make up a syndromic diagnostic entity". This chapter focuses only on the main aetiological, histopathological, clinical, diagnostic, and treatment features of MTLE-HS.

Aetiology and basic mechanisms

The epileptogenic potential of MTLE-HS is a result of a large variety of changes in the hippocampus, foremost being neuronal cell loss, proliferation of glial cells, and synaptic reorganisation. However, whether these changes are the causes or consequences of epileptic seizures, and the order in which they appear, have been issues of debate for decades.

Retrospective studies of surgically-treated patients have revealed a high occurrence of early childhood incidents. These events, also referred as to "initial precipitating incidents (IPI)", include febrile seizures, hypoxia, trauma, and intracranial infections. While identified in several retrospective studies, no prospective study yet exists that confirms these results (Mathern et al., 1995). A large number of mechanisms through which epileptogenesis may develop in MTLE-HS have been suggested. These include: glutamate neurotoxicity, mitochondrial dysfunction, immunological responses, genetic predisposition, and multiple acquired factors. To detail all these mechanisms would go far beyond the scope of this thesis. However, a fascinating story emerges from novel concepts that bring together glial dysfunction and disease development in neurological diseases. With regard to epilepsy, it is suggested that modified astroglial function may have an important role in the generation and spread

of seizure activity. To study this new idea of glia-mediated epileptogenesis is the primary objective of this thesis and is elaborated in detail in subsequent sections.

Main histopathological findings

The term *sclerosis* is based on the macroscopic finding of a shrunken, indurated hippocampus, and had already been described in the first quarter of the 19th century (Bouchet and Cazauvielh, 1825). The principal histological findings of HS can be dated back to the descriptions of Sommer and Bratz at the end of the 19th century, as previously mentioned (Sommer, 1880; Bratz, 1899). The histopathological hallmarks of MTLE-HS include segmental pyramidal neuron loss, glia cell proliferation, and dispersion of granular cells. The ILAE commission report on MTLE with HS (Wieser et al., 2004) proposed the typical pathological changes in HS, as listed here:

1.) As minimal criteria, neuronal loss, and gliosis predominantly involve CA1 and end-folium (CA3 and CA4) with relative sparing of the CA2 and subiculum.

2.) All hippocampal regions may show cell loss and gliosis to varying degrees.

3.) Functional and structural glial changes appear.

4.) Synaptic reorganisation often occurs.

5.) Dentate (granular cell) dispersion is encountered in more than 50 % of cases.

6.) Extrahippocampal pathology at other mesial temporal lobe structures, e.g. amygdala.

7.) Other pathological findings may be found, depending on which staining techniques are used.

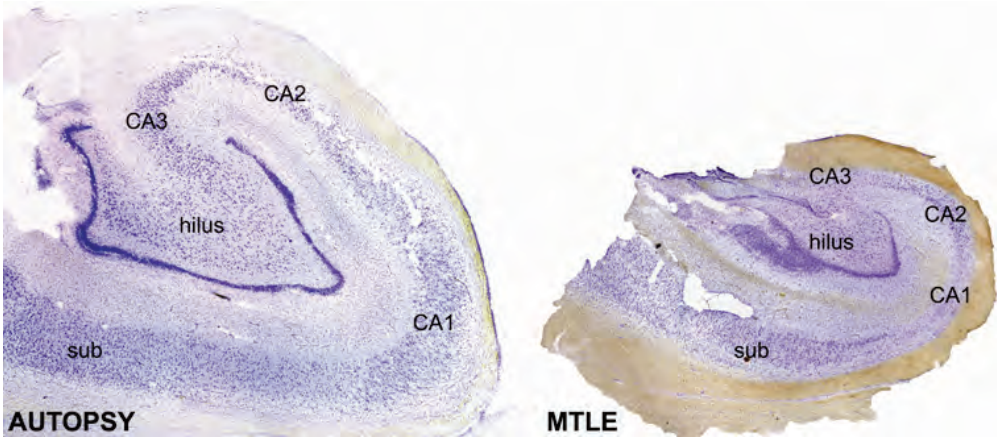


Figure 2: Nissl stained coronal sections of human hippocampus formations from autopsy (left) and from a patient with MTLE-HS (here referred to as MTLE, right)

The autopsy hippocampus shows a normal anatomical structure, whereas the MTLE hippocampus is smaller due to sclerosis and shows typical histopathological changes as loss of neurons, especially in CA1, glial proliferation, and granular cell dispersion in the hilar granular cell layer.

Clinical phenomenology

The archetypical medical history of patients with MTLE-HS starts with an IPI in early childhood, followed by a latent period of variable duration without any apparent clinical signs or symptoms, before habitual seizures occur. Habitual seizures often arise towards the end of the second decade of life, and tend to occur earlier in MTLE-HS than in other TLE/MTLE patients. (Davies et al., 1996; Janszky et al., 2004; Villanueva et al., 2005). Habitual seizures may initially respond to pharmacological treatment, but, over time, become more and more refractory (Semah et al. 1998). The progressive nature of this clinical presentation has been the subject of vigorous debate. Given that MTLE-HS worsens with time, could this process be affected or delayed by any kind of pharmacological or non-pharmacological treatment? Animal kindling models support a progressive course, as recurrent seizures lead to synaptic reorganisation and neuropathological changes in the hippocampus comparable with those of HS in humans (Sutula T et al., 1988). The degree of hippocampal atrophy may correlate with changes in magnetic resonance imaging (MRI), further supporting the worsening nature of the condition (Cendes F et al., 1993). However, evidence of HS is not necessarily related to seizure severity and may occur in individuals who

never experience seizures (Kobayashi et al., 2002). It is important to remember that benign forms of MTLE-HS also exist, which are readily controllable with common AEDs. Most studies, however, generate results from surgically treated MTLE-HS individuals, who represent the most pharmacoresistant cases.

Mood disturbance and cognitive impairment

The mesial temporal region participates in emotional expression and is implicated in mood disturbances. Interictal depression is a frequent psychiatric symptom in epilepsy and has been related to epilepsy of temporal origin (Mendez et al. 1986; Gaitatzis et al., 2004). However, whether depression occurs more frequently in MTLE-HS than in other TLEs is still a subject of debate. Some studies implicate depressive symptoms more frequently in MTLE-HS, while others find no evidence of a specific temporal target region for depressive mood (Quiske et al., 2000; Helmstaedter et al., 2004).

There is ample evidence of cognitive impairment in MTLE-HS. Typically, MTLE is associated with impairment of episodic memory, which means that retrieval of newly acquired information or consolidation of long-term memory is affected (Hermann et al., 1997). Some imaging studies confirm that the degree of memory disturbance correlates, to some extent, with the severity of hippocampal atrophy in MTLE-HS (Baxendale et al., 1998; Sawrie et al. 2001). Other determinants of memory disturbance are age of onset and severity of the epilepsy, additional extrahippocampal brain lesions (dual pathology), and use of AEDs. However, data concerning the latter aspects are too limited to reach any definitive conclusions.

In summary, MTLE-HS is most probably associated with mood disturbance and memory deficits. However, data assessment is difficult and determining whether these features are primary phenomena or secondary sequelae due to psychosocial aspects or use of AEDs, is problematic. Further studies are required to provide convincing evidence, and possible confounding factors have to be taken into account.

Seizure characteristics

Many descriptions of ictal and postical clinical signs and symptoms in MTLE have been published, and, for practical reasons, will be mentioned only superficially here.

MTLE is commonly associated with focal seizures (SPS and CPS, according to prior classifications). As there are no pathognomonic clinical signs, it is difficult to distinguish between seizures associated with MTLE-HS and seizures due to other MTLE, despite some studies that propose separability (Saygi S et al., 1994). Ictal characteristics can be divided into *subjective* and *objective* components.

Subjective epileptic events are referred to as *aura*. The majority of MTLE patients experience auras (French et al., 1993) Auras may occur as isolated phenomena (prior SPS), or occur as a first manifestation of focal seizures that involve larger networks (prior CPS) (Sperling et al., 1989). The most frequent aura in MTLE is characterised by visceral sensations, often experienced as a rising sensation in the epigastric region (Duncan et al., 1987; French et al., 1993). Other aura subtypes comprise anxiety, déjà vu and jamais vu, olfactory hallucinations, micropsia and macropsia, and feelings of depersonalisation.

Objective ictal manifestations of MTLE usually involve impairment of consciousness. These commonly begin with motor arrest, staring, and pupillary dilatation, before evolving to coordinated motor activities, referred as to *automatisms*. Automatisms are often of the oro-alimentary type (chewing, licking, lip-smacking, and tooth grinding), or present with stereotyped movements such as gesticulating and fumbling, or, less frequently, vocalisation, spitting, and bicycling movements.

Other objective manifestations, that may have some localizing and lateralizing value, and are often associated with seizure spread to suprasylvian brain areas, are head and eye deviation, unilateral or contra-lateral tonic or dystonic posturing (Kotagal et al., 1989), ictal vomiting (Kramer et al., 1988), unilateral eye blinking (Benbadis et al., 1996), and language disturbances such as aphasia, speech arrest, or vocalisation (Yen et al., 1996). Secondary generalisation occurs in MTLE, but usually shows variations of the classic tonic-clonic semiology (Wieser et al., 2004). Postictally, patients with MTLE often display visual relaxation, aphasia, confusion, and, exceptionally, motor deficits (Theodore et al., 1983).

The clinical phenomenology and seizure semiology of MTLE-HS form the basis for establishing the diagnosis. EEG and imaging is not a centrepiece of this thesis, and therefore will not be discussed here.

The diagnosis of MTLE-HS cannot be established on the basis of one or few criteria. It requires a constellation of signs and symptoms, together with EEG and imaging findings, neuropsychological data, and careful evaluation of patient history.

Treatment and outcome

To the author's knowledge, no systematic comparative studies on the pharmacological treatment of MTLE-HS have been conducted. Medical treatment, therefore, includes most of the current AEDs, preferably those with documented effect on focal seizures, such as carbamazepine, oxcarbamazepine, lamotrigine, levetiracetam and topiramate, but also valproate, phenytoin, phenobarbital, zonisamide, and others. The efficacy of a specific AED at controlling MTLE-HS may last for several years, until the condition becomes increasingly refractory (Berg et al., 2003). The diagnosis of MTLE-HS is often not established until the patient attends a tertiary centre due to the lack of effect from medication. For this reason, no definitive information exists on the proportion of patients whose condition remains adequately controlled by pharmacotherapy.

For medically resistant patients, surgical anteromesial temporal lobectomy is the treatment of choice and has been proven successful (McIntosh et al. 2001; Engel J Jr et al., 2003) Surgical treatment of patients with TLE has been reviewed extensively with particular focus on predictors of outcome (McIntosh et al., 2001). MTLE-HS has been identified as having a better surgical outcome than other forms of TLE (Berkovic et al. 1995; Lee et al., 1998). Moreover, a history of febrile convulsions in childhood has been reported to be significantly associated with a good seizure control outcome (Wieshmann et al., 2008). Febrile seizures are most likely to be associated with MTLE-HS, as elaborated in subsequent sections.

1.1.6 Febrile seizures

Febrile seizures (FS) are the most frequent type of convulsions in childhood. A febrile seizure is defined as "an event in infancy or early childhood, usually occurring between three months and five years of age, associated with fever but without evidence of intracranial infection or other defined cause" (NIH, 1980). FS should be distinguished from epilepsy, which is defined by recurrent *non-febrile* seizures.

FS are usually grouped into *simple* and *complex* types. Simple FS are short, non-recurrent, generalised tonic-clonic seizures of less than 15 minutes duration. Simple FS account for approximately 75 % of all FS and occur, *per definitionem*, before the 6th birthday. Complex FS are usually prolonged and often recur up to several times within a 24-hour period. They may lead to focal or generalised seizures or even to febrile status epilepticus (SE) (Jones et al., 2007)

The cumulative incidence of FS shows regional variation, with 2-5 % in Europe and USA, 6-9 % in Japan, and, the highest, 14 % in Guam in the Pacific Ocean (Stanhope et al., 1972; Tsuboi et al., 1984; Hauser et al., 1994; Hauser et al., 1996) These regional epidemiological differences strongly indicate a genetic propensity for the occurrence of FS (Hauser et al., 1985; Tsuboi et al. 1991; Vestergaard et al. 2002). This is supported by family and twin studies that have shown an increased risk for relatives, in comparison with the general population (Tsuboi, 1977; Tsuboi et al., 1991). Either a polygenetic inheritance mode or autosomal dominant inheritance with incomplete penetrance has been discussed (Rich et al., 1987; Johnson et al., 1996).

Although no specific genes that account for the majority of FS cases have been identified, family linkage studies have, to date, identified a total of 9 genetic loci (FEB1-9) for FS susceptibility (Nakayama et al., 2009). Furthermore, a number of association studies have been conducted and revealed 7 genes related to this condition (Nakayama et al., 2009; http//www.epigad.org). However, most association studies do not fulfil stringent criteria, are statistically underpowered, or lack replication (Tan et al., 2004; Nakayama et al., 2009).

A genetic predisposition accounts for the highest predictive factor for the development of FS. However, a variety of other risk factors have been identified, including low plasma ferritin levels (Pisacane et al., 1996), high fever, retarded development and low birth weight (Vestergaard et al., 2002), and HHV6 or influenza A infections (Milichap et al., 2006; Suga S et al. 2007). The pathophysiology of FS is unknown. As febrile seizure susceptibility is highest in young children and decreases significantly with advancing age, developmental factors probably play an important role (Jensen and Baram, 2000).

1.1.7 Febrile seizure-related epilepsies

Generalised epilepsy with febrile seizures + (GEFS+)

GEFS+ is an autosomal dominant familial syndrome with high penetrance, characterised by heterogeneous, mainly generalised, seizure phenotypes (Scheffer et al., 1997). Febrile seizures often persists beyond the usual age of 6 years (FS+), and patients may present with afebrile, generalised or focal seizures, and cases associated with severe myoclonic epilepsy in infancy (SMEI) have also been described. Different gene mutations have been identified, three of which involve subunits of the voltage gated sodium channels, SCN1 and SCN2, and two within

genes encoding subunits of the $GABA_A$ receptor (Wallace et al., 1998; Escayg et al., 2000; Sugawara et al. 2001; Baulac et al., 2001; Dibbens et al., 2004). However, screening for these genes in FS populations has failed to detect an association (Nakayama et al., 2003).

Severe myoclonic epilepsy in infancy (Dravet syndrome)

SMEI is a serious epileptic syndrome that arises in the first year of life, often with prolonged, focal seizures often preceded by a relatively mild fever. SMEI is associated with developmental delay at around 1 year of age, accompanied by other afebrile seizure types, such as myoclonic, atypical absences, as well as ataxia and behavioural problems. SMEI may be analogous to GEFS+, and the discovery of mutations in the same SCN1A channel genes supports this theory (Ohmori et al., 2002; Wallace et al., 2003).

1.1.8 Association between MTLE-HS and febrile seizures

About 13 % of all patients with epilepsy experience FS in childhood, with large variation between the different epilepsy syndromes (Hamati-Haddad et al., 1998). The strongest association has been postulated to exist between FS and TLE (25 %), especially with refractory MTLE-HS (50-80 %) (French et al., 1993; Maher et al., 1995). This strong association supports the theory that FS may lead to hippocampal injury and subsequent unprovoked seizures. However, whether FS precipitate HS, or vice versa, (i.e., hippocampal alterations lead to FS), has been a key question for several decades.

Retrospective studies of patients with TLE, and particularly with MTLE-HS, usually show a high association with FS in infancy (Cendes et al., 1993; French et al., 1993).

A large variety of prospective studies, including many MRI studies, have been conducted to determine whether FS precipitate hippocampal injury and subsequent MTLE-HS. Most prospective studies do not confirm a link between FS and MTLE-HS (Camfield et al. 1994; Tarkka et al.; 2003), but other studies indicate the opposite (Farrow et al., 2006; Vestergaard et al. 2007).

Lately, data from genetic studies have contributed to reinforcing a link between FS and MTLE-HS. A high incidence of FS in familial MTLE supports a common genetic basis (Berkovic et al. 1996; Hedera et al., 2007). Moreover, patients with TLE and a history of FS (TLE-FS) have a higher frequency of first grade family members with FS

(Briellmann et al., 2001), and mutations have been associated with simple FS, hippocampal abnormalities, and TLE (Colosimo E et al., 2007).

Animal studies have been important tools for investigations of whether FS have the potential to result in epilepsy in the absence of genetic or acquired predisposing factors. A large variety of animal studies have shown that rodents exposed to hyperthermia develop spontaneous unprovoked seizures and pathological changes in the hippocampus that are comparable to those in patients with MTLE-HS (Dube et al., 2010; Scantlebury et al., 2010; McClelland et al., 2011).

However, there is no evidence for the theory that complex FS imperatively leads to hippocampal alteration and subsequent MTLE, and a simple equation should be considered by those who advocate adoption of this idea:

2-6 % of children in western countries develop febrile seizures, of which complex FS account for a fraction (approximately 15 %), suggesting that the incidence of complex FS is 1:300. However, MTLE-HS is significantly less frequent. The relationship between FS, hippocampal sclerosis, and the development of MTLE is complex, and probably depends upon multiple factors, most of which are still elusive.

1.2 Genetics of temporal lobe epilepsy

Until the mid 1990s the majority of clinicians and scientists considered TLE to be an acquired disorder. However, recent evidence has refuted this concept and today we know that genetic factors play an important role.

1.2.1 Historical notes

In 400 BC, Hippocrates wrote the following on epilepsy in his manuscript *On the Sacred Disease*: “It begins to be formed while the foetus is still *in utero*” (Page TE et al., 1967). Taking into account that general medical knowledge was still in its infancy, this statement could be considered the first noted recognition of inherited components in epilepsy. Unfortunately, the realisation that epilepsy can be passed from one generation to the next, led to the implementation of marriage restrictions for people suffering from epilepsy. Even as recently as December 1999, India for example had a law regarding epilepsy and marriages in its *Hindu marriage act*, constituting that marriage could only be solemnised “if, at the time of marriage, neither party suffers from recurrent attacks of insanity or epilepsy”.

A pioneer of the “modern” epilepsy genetics field was the American neurologist and former president of the ILAE, William Gordon Lennox (1884-1960). Lennox started investigating twins and families with epilepsy in the mid-1930s and his enthusiastic recruitment of twin pairs with epilepsy, resulted in the publication in 1960 of his classic manuscript entitled “The genetics of epilepsy” (Lennox and Lennox, 1960) With his assembly of 225 twin pairs, Lennox was able to prove the existence of heritable genetic factors in epilepsy. The notably higher concordance rate in monozygotic, compared with dizygotic twins in different epilepsy phenotypes was probably his most important observation (Vadlamudi et al., 2004). Lennox also understood that genetic factors alone could not explain his findings, and he anticipated the presence of exogenous factors, in conjunction with heritable factors, being of causative importance in epilepsy. With his observations, Lennox paved the way for the “multifactorial concept of inheritance” of the epilepsies, which is still alive today.

1.2.2 Genetic classification of the epilepsies

Classifications that attempt to encompass the genetic background of the epilepsies commonly apply the following categorisation (Johnson et al., 2001; Reid et al., 2009):

1) Epileptic seizures arising in the context of multi-organ hereditary disorders. These include chromosome disorders, neurocutaneous disorders, neurodegenerative disorders, genetic disorders of cortical development, and a large assortment of metabolic diseases.
2) Idiopathic epilepsies with simple Mendelian inheritance. These are rare (1-2 %) epilepsies, based on a single mutant gene, and can be passed on to the next generation in several ways: autosomal dominant or recessive, X-linked dominant or recessive, Y-linked or mitochondrial. Most mutations in epilepsies have been found in different ion channel genes.
3) Idiopathic epilepsies associated with cytogenetic (chromosomal) abnormalities. These are epilepsies based on *de novo* gene mutations, without heritable factors.
4) Epilepsies with complex inheritance, involving multiple contributing factors. These epilepsies account for at least 50 % of all epilepsies, and include most forms of idiopathic generalised epilepsies, as well as partial epilepsies, and also encompass the TLE.

All these categories may, in the broadest sense, include TLE cases. However, most TLE cases involve complex inheritance patterns and, therefore, belong in category 4. However, there is a growing list of several forms of familial temporal lobe epilepsies (FTLE) that could, at least in part, be consistent with category 2. For practical reasons, FTLE will be discussed here first.

1.2.3 Familial temporal lobe epilepsy (FTLE)

A family history of epilepsy is not uncommon in patients with TLE. However, familial TLE do not comprise a single syndrome. Different forms of familial TLE exist, and it is crucial to conduct a detailed family anamnesis in order to define the familial epilepsy syndrome. Familial forms of TLE are now included in the latest classification of epileptic syndromes by the ILAE (Berg et al., 2010). Familial and sporadic (non-familial) TLE cannot be distinguished on the basis of their clinical presentations, as there are no specific phenotypic characteristics. Hence, the family history has to be apparent. In the absence of any other suspected generalised or dominant partial epilepsy, at least two family members have to be affected to for a diagnosis of a familial form of TLE to be established.

FTLE can be subdivided into two main, genetically distinct syndromes: mesial (FMTLE) and lateral/neocortical (FLTLE), depending on seizure onset, semiology, and MRI features.

Familial lateral temporal lobe epilepsy (FLTLE)

FLTLE is also termed autosomal-dominant partial epilepsy with auditory features (ADPEAF), and was first described by Ottman et al. in 1995 (Ottman et al., 1995). Clinically, this condition presents with auditory auras, characterised by ringing or humming sounds, followed by early ictal aphasia. Onset is in the first three decades of life. There is no association with febrile seizures, and MRI is usually normal. The course of the disease is benign (Winawer et al., 2000). Linkage analysis has revealed a locus on chromosome 10q24, and this later emerged as the locus of the leucine rich glioma-inactivated gene 1 *(LGI1)* (Kalachikov et al., 2002). Similar families, mapping to the same region, have been described by Norwegian epileptologists, but with a somewhat different clinical picture, with prominent visual symptoms and sensory dysphasia due to lateral temporal lobe origin (Poza et al., 1999; Brodtkorb et al., 2002). To date, a total of 25 mutations in *LGI1* associated with FLTLE have been identified (Nobile et al., 2009).

LGI1 was initially described as a candidate tumour suppressor gene for glioma (Senechal et al., 2005), but more recently was identified as a subunit of the presynaptic Kv1 voltage gated potassium channel, preventing its inactivation (Schulte et al., 2006). LGI1 has been isolated from the brain where it serves as a ligand for two epilepsy-related receptors, ADAM22 and ADAM23 (Fukata et al., 2010). An

implication in epilepsy has been proved by loss of LGI1 in mice (LGI1 -/-), which causes lethal epilepsy. Interestingly, LGI1 has very recently been identified as an autoantigen, associated with limbic encephalitis/autoimmune synaptic encephalopathy (Lai et al., 2010), a condition often associated with seizures and neuropsychiatric symptoms. To address this issue in detail would, however, go beyond the scope of this thesis.

Familial mesial temporal lobe epilepsy (FMTLE)

A familial form of TLE with mesial seizure onset (FMTLE) was first recognised in twin studies by Berkovic et al (Berkovic et al., 1994; Berkovic et al., 1998), as a result of the observation of high concordance rates in monozygotic twins compared with dizygotic twins, and was reinforced by observations in non-twin families (Berkovic et al., 1996).

Although there seems to be no clear cut consensus in the literature regarding sub-classification of FMTLE, three subtypes are currently considered (Gambardella et al., 2009): benign FMTLE without HS or FS, FMTLE associated with HS, and FMTLE associated with FS.

Benign FMTLE without hippocampal sclerosis or febrile seizures

Patients in this category of FMTLE typically present with early adulthood epilepsy onset, and with auras including psychiatric (predominantly déjà vu and jamais vu) and autonomic features (Berkovic et al., 1996). CPS and secondary generalisation are rare in this phenotype, and EEG recordings often do not show any epileptic activity. Patients have no signs of HS in MRI, and no history of childhood FS. The course of this TLE subtype is benign and the prognosis considered excellent, and therefore this condition is probably under-diagnosed. Despite the report of a probable linkage to chromosome 4q in a single pedigree (Hedera et al., 2007), the genetic basis of FMTLE is largely unknown.

FMTLE associated with HS

The identification of FMTLE associated with HS and/or FS, has altered the view that hippocampal sclerosis is exclusively associated with sporadic/ acquired forms of TLE. In 2003, Kobayashi et al. first described a relationship between FMTLE, HS and FS,

with large intrafamilial and interfamilial phenotypic heterogeneity with respect to severity of the epilepsy, history of FS, and presence of HS (Kobayashi et al., 2003).
In 2/3 of families, the course is rather mild, while 1/3 present with severe, treatment-refractory seizures. Approximately 10 % of patients have a history of FS, and the mean age of epilepsy onset is 10 years. Seizure semiology frequently includes CPS, with oro-alimentary automatisms, and, postictally, patients often are plagued by confusion. In accordance with benign FMTLE, secondary generalisation is rare. MRI reveals a broad variation in HS, from mild to severe, and the severity of hippocampal abnormalities have been reported to show a relationship with the clinical picture (Kobayashi et al., 2003).
Interestingly, MRI findings of HS have also been observed in asymptomatic family members, which suggest that the HS itself might be inherited, rather than that the epilepsy leads to hippocampal alteration (Kobayashi et al., 2003). Histological studies of the HS in this subgroup of FMTLE patients have not shown any differences from sporadic MTLE. Taken together, these observations indicate that it is highly probable that at least some HS is based on a complex interaction between genetic and environmental factors.

FMTLE associated with FS

To date, reports have been published regarding two large FMTLE family clusters of FMTLE associated with FS (Baulac et al., 2001; Claes et al., 2004). The phenotype of the FMTLE in members of both families was characterised by onset before age of 20 years, and none of the family members show signs of HS by MRI. The clinical course is benign, and, typically, the predominant clinical feature in members of these families is FS, while TLE occurs less often. Digenetic inheritance was proposed for one of the families, with gene loci on *18qter* and *1q25*-31 (Baulac et al., 2001), whereas in the second family linkage on *12q22-23.3* was shown (Claes et al., 2004).
Other variants of FMTLE associated with FS have been demonstrated by identification of sodium channel mutations (SCN1A and SCN1B), which usually cause childhood febrile seizures, and rather infrequent MTLE and HS in a few family members (Scheffer et al., 2007; Colosimo et al. 2007).
These should not be confused with the syndrome GEFS+, which is another epilepsy phenotype associated with sodium channel mutations, and is discussed later in this thesis.

It is important that this summary regarding our knowledge on familial forms of TLE, does not give the impression that TLE typically occurs in families. These familial syndromes account for only a fraction of TLE cases, and the TLE phenotype usually appears sporadically, without any indicators of inheritance. However, the growing list of family studies and increasing knowledge about the aetiology of complex diseases suggest that genetic factors are, indeed, involved in TLE and provide a good reason to move our focus away from the paradigm that TLE is merely an acquired disorder.

TLE are complex disorders, in which a plethora of genes are probably involved, affected by environmental factors, and complicated further by post-transcriptional modifications. In order to learn more about the aetiology and pathogenesis of TLE, which is a prerequisite for establishing cures for patients, it is important both to broaden the spectrum of genetic tools used for investigation and, at the same time, dissect the sub-groups of the TLE, as based on phenotypic characteristics.

Linkage analysis in large family pedigrees indicated autosomal dominant inheritance for the syndromes described above. However, as the majority of TLE cases, do not occur within families, linkage analysis has clear limitations. In this context, it should be noted that genetic variants may contribute to increased susceptibility to development of TLE. Identification of susceptibility genes can be performed by population-based association studies using candidate gene approaches. This has been one of the major tasks of this thesis, and is expounded in detail in subsequent sections.

1.2.4 Genetic association studies in TLE

Genetic changes may contribute to disease development in humans. On the one hand, rare mutations may occur that have extensive effects on the phenotype, typically leading to monogenic diseases. On the other hand, common genetic variants with limited phenotypic effects might also result in disease (Cordell et al., 2005). A single nucleotide polymorphism (SNP) is a DNA sequence variation occurring when a single nucleotide (adenine, guanine, cytosine, or thymine) in the genome differs between members of the same biological species. These variations in the DNA sequence can affect the development of diseases.

For a variation to be considered a SNP, it should occur in at least 1 % of the population. SNPs can occur in coding (gene) and non-coding regions of the genome.

As well as SNPs, other modifications in the genetic information may influence phenotype and confer disease susceptibility. These include rare variants with occurrence < 1% in the general population, copy number variations, and epigenetic modifications. The latter will be discussed later in more detail.

Genetic association studies are designed to compare the frequency of specific alleles (in the narrow sense SNPs) in affected cases with those in unaffected control subjects. An allele is said to be associated with the disease when its frequency differs between cases and controls more than would be predicted by chance (Lander et al., 1994).

A large number of genetic association studies have been conducted on different epilepsy entities, candidate genes, and populations (Hirschhorn et al., 2002). Until recently, there was lack of systematic databases summarizing which candidate genes in which epilepsies have been investigated. This gap has been filled by the establishment of an online repository of data relating to genetic association studies in epilepsies, the Epilepsy Genetic Association Database (http://www.epiGAD.org), which is supported by the ILAE Genetics Commission. To date, the database contains 212 susceptibility gene studies, of which approximately 25 % are related to TLE. The most important and most widely discussed susceptibility genes in TLE are summarised in table 1.

Table 1: Summary of the most relevant genes tested for association with TLE

Gene	Number of studies (positive/negative)	Allele	Relevant studies	Postulated function
Interleukin-1β	6 (2/4)	C511T	- Kanemoto et al., 2000 - *Heils et al.,2000* - *Buono et al., 2001*	- Proinflammatory cytokine. - Interleukin receptors found in the hippocampus
Prodynorphin	10 (4/6)	L-allele	- Stögmann et al., 2002 - Salzmann et al., 2008 - *Cavalleri et al. 2005*	- Opioid polypeptide hormone. - Involved with chemical signal transduction - Endogenous anticonvulsant?
Apolipoprotein E	8 (3/5)	ε4	- Briellmann et al., 2000 - *Cavalleri et al.,2005*	- Involved in catabolism of triglyceride-rich lipoprotein constituents
GABA (B) receptors	9 (2/7)	G1465A	- Gambardella et al., 2003 - *Cavalleri et al., 2005*	- Metabotropic GABA receptors - hyperpolarizing neuron via opening of K+ channels
Prion protein (PRNP)	5 (2/3)	N171S	- Walz et al., 2003 - *Cavalleri et al., 2005*	- Express *major prion protein* - unknown function

Negative association studies in italic font; source: Tan et al., 2004 and http://www.epiGAD.org

1.2.5 Genome-wide association studies in TLE

The first genome-wide association study (GWAS) in the epilepsies has only recently been published (Kasperaviciute et al., 2010). This large GWAS included 3445 patients with partial epilepsies, of which 919 were diagnosed with mesial temporal lobe epilepsy. No significant genome-wide association was identified, leading to the conclusion that the genetic architecture of the partial epilepsies is likely to be highly complex. The consortium behind this study has suggested establishing further association studies, including GWAS, but in more homogenous and narrowly-defined

cohorts, with sample sizes that generate adequate power. This is challenging for several reasons, but particularly because population stratification may evolve when large samples are collected from different geographical regions.

1.2.6 Genetic association studies carried out in this thesis

Most genetic association studies involving TLE populations have not provided compelling results (Tan et al., 2004). One reason for this is that the TLE are genetically complex disorders, probably influenced by variation in several susceptibility genes. Another challenge is selection of appropriate candidate genes that have a high degree of biological plausibility.

Many of the candidate genes investigated in epilepsies are those encoding *neuronal* ion channels or receptor proteins, based on the concept that alterations in these substrates may contribute to changes in membrane potential and lead to *neuronal* hyperexcitability.

A rather newer concept is that altered *glial* function may also play an important role, and lead to hyperexcitability of neuronal tissue. Astrocytes, in particular have been suggested to promote epileptogenesis and disease progression in epilepsy and other neurological conditions (Binder and Steinhäuser, 2006; Seifert G et al., 2006, Eid et al., 2008). Further details on the concepts of glia mediated epileptogenesis are elaborated in section 1.3. This thesis describes 3 novel candidate genes investigated in 2 different association studies. The first association study (Paper II) focussed on the glial target genes *AQP4* and *KCNJ10*, and the second study (Paper III) had the extracellular matrix enzyme, matrix metalloproteinase-9 gene, *MMP-9* as its subject.

Genetic association study with *AQP4* and *KCNJ10* as candidate genes

It has been recently demonstrated that ion homeostasis in the brain depends not only on proper ion channel function, but also on water transport, mediated by specific water channels. In particular, deletion of the glial water channel aquaporin-4 (AQP4) or its anchoring protein, alpha-syntrophin, interferes with K^+ clearance from the extracellular space (ECS) after high frequency stimulation in slices or *in vivo* (Amiry-Moghaddam et al., 2003; Binder and Steinhäuser, 2006), as well as after mechanically-induced spreading depression (Padmawar et al., 2005). The finding that AQP4 is co-localised with the inwardly rectifying K^+ channel Kir4.1 (Nagelhus et al., 1999) led to the suggestion that AQP4 and Kir4.1 form a coupled water and K^+

transport unit (Amiry-Moghaddam et al., 2003; Nagelhus et al., 2004). The expression of this unit is compromised in TLE, as judged by the partial loss of AQP4 and its anchoring complex from astrocyte endfoot membranes in the hippocampi of patients suffering from this condition (Eid et al., 2005).

As deficiencies in K^+ clearance would be expected to perturb neuronal excitability and increase the propensity to seizures, we investigated whether polymorphisms occur in those genes encoding AQP4 and the potassium channel Kir4.1 that are associated with TLE and its subgroups MTLE-HS and/or TLE-FS. To this end, we resequenced *AQP4* to identify new SNPs in the TLE subgroups and also included known SNPs within *AQP4* and *KCNJ10* from HapMap (http://www.hapmap.org) and dbSNP (http://www.ncbi.nlm.nih.gov) databases.

The timeliness of this study was underscored by previous data that suggest a possible association between variations in the human *KCNJ10* gene and seizure susceptibility (Buono et al., 2004; Lenzen et al., 2005), and mutations in the *KCNJ10* gene, that are associated with the EAST/SeSAME syndrome, characterised by epilepsy (Bockenhauer et al., 2009; Scholl et al. 2009).

Genetic association study with *MMP-9* as candidate gene

One elemental characteristic of the human brain is its capacity to undergo lifelong morphological and functional changes. These processes, also referred to as remodelling, include cortical reorganisation, synapse formation, and neurogenesis. Remodelling occurs during brain development and learning, but also serves as an adaptive mechanism to compensate for lost function (Bruel-Jungerman et al., 2007; Parent, 2007; Eisch et al., 2008). Studies of the hippocampus have identifed a relationship between synaptic remodelling and epilepsy. (Parent, 2007; Abrous et al., 2005; Kempermann et al., 2004). In TLE, especially in cases with HS, remodelling may result in defective synaptic rearrangement of neuronal circuits and thus promote epileptogenesis and disease progression (Pitkanen and Lukasiuk, 2009).

A relatively recent discovery is the identification of matrix metalloproteinase-9 (MMP-9) as a possible key factor in the development of aberrant synaptic plasticity and dendritic pruning in animal models of TLE (Wilczynski et al., 2008)

MMP-9 is a member of the family of the matrix metalloproteinases (MMP), which constitute zinc-dependent extracellular or membrane-bound endopeptidases. Their

primary function is cleavage of extracellular matrix (ECM) proteins, and thus they are involved in processes of physiological tissue remodelling (Werb, 1997).

MMP-9 has been implicated in various central nervous system (CNS) pathologies including stroke (Park et al., 2009), traumatic brain injury (Hayashi et al., 2009), cerebral arteriovenous malformations (Chen Y et al., 2008) influenza-associated encephalopathy (Ichiyama et al., 2007), systemic lupus erythematosus with CNS affection (Trysberg et al. 2004), and in meningitis, where it has been identified as a risk factor for developing neurological sequalae (Leppert et al., 2000).

In addition, recent studies have indicated a physiological role for MMP-9 in neuronal plasticity, including learning and memory, as well as long-term-potentiation (Nagy O et al., 2006). It has been suggested that MMP-9 may have an initial detrimental effect, leading to neuronal cell loss, but also a subsequent beneficial (restorative or neuroprotective) effect (Zlokovic, 2006; Michaluk and Kaczmarek, 2007).

Regarding a possible role in epileptogenesis, MMP-9 is believed to cleave extracellular matrix molecules in and around the synaptic cleft. Thus, MMP-9 activation may be an essential step in the cascade of events leading to new synapse formation, and therefore could be critical for the sequence of events that underlies the development of seizures.

Transgenic rats that over-express MMP-9 have been found to develop increased susceptibility to seizures, whilst deletion of the *MMP-9* gene in mice leads to alleviation of seizures (Wilczynski et al., 2008).

Based on these findings, we hypothesised that particular polymorphisms of the *MMP-9* gene could contribute to the development of TLE, or subgroups of this condition, notably MTLE-HS and TLE-FS.

Despite a broad selection of SNPs in the *MMP-9* gene and strong adherence to the general guidelines for establishing genetic association studies, we were unable to find any association with TLE or its subgroups. However, the possibility that TLE is associated with changes in *MMP-9* expression or regulation could not be ruled out and should be the subjects of further research.

1.3 Alterations of glial cell function in temporal lobe epilepsy

1.3.1 Glia – research milestones

Glia cells (from glia, Greek for “glue”), also termed neuroglia or simply glia, are non-neuronal cells and represent the most numerous cell type in the CNS. Glia was first described by the German doctor and pathologist Rudolf Ludwig Karl Virchow (1821 – 1902), whom, contrary to the accepted dogma of the time, argued that the brain contains connective tissue. In a series of papers published in 1856 and his textbook (1858), Virchow introduced the term *Nervenkitt*, later translated to nerve-glue or glia (Virchow, 1862, Somjen, 1988).

Glial cells are subdivided into three main groups: *Oligodendroglia*, which are involved in the production of myelin and “insulation” of nerve cell axons; *microglia*, which are part of the CNS immune system; and *astroglia* or *astrocytes*, which were thus named due to their characteristic star shape by the Spanish neuroscientist and pathologist, Ramón y Cajal (1852 – 1934).

For decades astrocytes have been considered as relatively passive cells, the existence of which was mainly justified by their role as “servants to neurons”. They were considered to have three functions: i) to act simply as glue holding nerve cells in place, ii) to be involved in scar formation in the CNS, and iii) to have a nutritive function in relation to neurons. They were regarded as electronically silent and deficient in ion channels and transmitter receptors.

In recent years, this view has altered radically. We have learned that astrocytes form an intimately connected network with neurons, and serve as active communication elements with a large variation of integrative functions in the CNS. Knowledge about astrocytes is crucial for understanding normal brain functioning, and astrocytes provide a promising new area for treatment of neurological diseases, including epilepsy.

Before elaborating further on astrocyte function and dysfunction, it is important to note that astrocytes are not one single cell type. Quite the contrary, different cells with astroglial properties exist both intermingled in a given brain region, and, with varying distribution, in different brain areas. To date, classification into two main astrocyte cell types has been established. These are termed GluT cells and GluR cells, based on their segregated expression of glutamate transporters (GluT cells)

and ionotropic glutamate receptors (GluR cells), respectively. These astrocyte subtypes have dissimilar electrophysiological and anatomical properties, and show diverse immunoreactivity to different markers (Jabs R et al., 2008). Although both cell types are referred to as astrocytes, their functional impacts should be considered to differ. However, which separate tasks the respective astrocyte subtypes perform is incompletely understood.

Astrocytes express a large range of ion channels and receptors, comparable with those from neurons (Verkhratsky and Steinhäuser, 2000; Seifert et al., 2004). However, the "quality and quantity" of receptors and channels varies between neurons and astrocytes. Neurons, for example, show a strong expression of Na^+ channels and a relatively weak expression of K^+ channels, while in astrocytes, K^+ channels greatly predominate.

One of these channels is Kir4.1, which belongs to the family of inwardly rectifying potassium channels. Kir.4.1 is co-localised with the astrocyte water channel AQP4 in glial cells (Nagelhus et al., 1999) and it has been suggested that AQP4 and Kir4.1 channels work in concert to maintain K^+ and water homeostasis in the CNS (Nagelhus et al., 1999; Nagelhus et al., 2004, Soe et al., 2009). Notably, deletion or mislocalization of AQP4 delays K+ clearance from activated synapses and increases severity of seizures (Amiry-Moghaddam et al., 2003; Binder et al., 2006). As these channels are a major focus of this thesis, their roles in epilepsy are discussed in more details in subsequent sections.

In contrast with mature neurons, astrocytes are usually coupled in networks of gap junctions, mainly formed by connexins 30 and 43, through which astrocytes can "communicate" with each other and dissipate molecules like K^+ and glutamate (Theis et al., 2005). Deletion of these connexins delays K+ clearance from activated synapses (Walraff et al., 2006). Gap junctions also contribute to the transmission of intercellular Ca^{2+} waves in astrocytes, which may be important for release of transmitters such as glutamate.

A further crucial function of astrocytes is the clearance of transmitters released by active neurons. Astrocytes express glutamate transporters (EAAT1 and EAAT2; in rodents termed GLAST and GLT-1), which take up glutamate from the ECS, and thus prevent glutamate accumulation that might otherwise lead to excitation and/or excitotoxicity (Bergles et al., 1999; Danbolt et al., 2001).

Another feature of astrocytes, which is still somewhat controversial, is the property that is currently subsumed in the concept of “gliotransmission”. Gliotransmission is the ability of astrocytes to release chemical transmitters, including glutamate and ATP. This ability was first described in a classical study by Parpura and colleagues in 1994, in which glutamate release from astrocytes was demonstrated in astrocyte-neuron co-cultures (Parpura et al., 1994; Volterra and Meldolesi, 2005). Following this groundbreaking study, a series of further studies demonstrated that gliotransmitter release is probably dependent on an increase in intracellular Ca^{2+} concentrations in astrocytes. The release was proposed to be orchesterated by G-protein coupled neurotransmitter receptors, which, via phosphorylase C, effect release of Ca^{2+} from internal stores in astrocytes (Haydon, 2001). Indeed, this proposal has been strongly supported by several studies that have shown that stimulation of neuronal afferents induces an elevation in Ca^{2+} concentrations within astrocytes (Porter et al., 1996), which then can spread to adjacent astrocytes (Charles et al., 1991). This means that astrocytes comprise a chemical form of excitability that is linked to neuronal activity in a bidirectional fashion (Haydon, 2001). Calcium-dependent transmitter release is a mechanism that, until recently, was considered to be only available to neurons.

It is still unclear which functions are involved in these astrocyte mechanisms, but it does seem certain that astrocytes have the ability to influence and modulate neuronal activity (Angulo et al., 2004). On the basis of these various interactions and effects associated with astrocytes, it seems plausible that altered astrocyte function may contribute to disease development and progression in different neurological diseases, including epilepsy.

1.3.2 Astrocytes and epilepsy

Current antiepileptic treatment concepts are mainly based on the principle that epileptic seizures arise from abnormal excessive or synchronous *neuronal* activity in the brain (Rogawski et al., 2004). Phenobarbital, for example, is an effective GABA receptor agonist that increases the efficacy of inhibitory synapses. Another example is phenytoin, a sodium channel blocker that reduces the rate and/or amplitude of action potentials in neurons, and hence inhibits high-frequency neuronal firing. Valproate attenuates excitability via a combination of several mechanisms, as

reviewed in (Johnston, 1984; Löscher, 1993), all of which are believed to have an impact on neuronal functions alone.

While 2/3 of all epilepsy patients respond successfully to currently available AEDs, the remaining 1/3 continue to suffer recurrent seizures, despite *per se* optimised pharmacological treatments (Kwan and Brodie, 2000). These pharmacoresistant cases are mainly represented by patients suffering from TLE. In order to fulfil the unmet medical needs of patients with pharmacoresistant TLE, alternative pharmacological treatment strategies should be a goal of epilepsy researchers and epileptologists. During recent years, evidence has accumulated that indicates that proper neuronal function is not possible without glial cells. Glial cells, especially astrocytes, are critically involved in the maintenance of homeostasis for ions and water, and hence in the pathophysiological mechanisms that lead to neuronal hyperexcitability and epilepsy. This makes glial cells an exciting subject for novel treatment concepts in epilepsy, especially for pharmacoresistant TLE (Jabs et al., 2008).

The hippocampal seizure focus in TLE has been the subject of most studies on the aetiology and pathogenesis of the epilepsies. The rationale for this is the need for novel treatment strategies for patients with TLE due to high pharmacoresistance. In addition, the availability of tissue from surgically-treated TLE patients and the wealth of experience on use of animal models of TLE are further reasons for extensive research in this field.

1.3.3 Roles of the inwardly rectifying potassium channel Kir4.1

Glial cells are characterised by strongly negative resting membrane potentials and highly selective membrane permeability to potassium ions (K^+) (Kuffler et al., 1966). These fundamental properties are maintained by profuse expression of K^+ channels, of which inwardly rectifying K^+ (Kir) channels predominate. Kir channels are responsible for the main K^+ conductance and sustain the resting membrane potential of glial cells close to the equilibrium potential of potassium, as demonstrated by a variety of electrophysiological studies (Sontheimer et al., 1994; Verkhratsky and Steinhäuser, 2000).

In contrast with the vast majority of K^+ channels, Kir channels are characterised by their ability to conduct more efficiently inwards than outwards. They are reliant on the

outward K^+ concentration [K+]out and are modulated by intracellular factors and secondary messengers (Doupnik et al., 1995).
The Kir channel family is generally divided into seven subfamilies (Kir1.0 - Kir7.0), with a total of between 16 and 20 members (Nichols et al., 1997; Olsen and Sontheimer, 2008).
Kir channels form tetrameric structures, assembled from homomeric or heteromeric Kir subunits, which implies further functional diversity (Krapivinsky et al., 1995). Based on different biophysical properties, Kir channels segregate into different subtypes (Butt et al., 2006). Glial cells may express all subtypes of Kir channels. However, the most abundant Kir channel in glial cells, and functionally the most important by far, is Kir4.1, which belongs to the subtype of ATP-dependent Kir channels.
Kir4.1 has been detected in astrocytes, oligodendrocytes, cerebellar Bergmann glia cells, and retinal Müller cells (Takumi et al., 1995; Ishii et al., 1997; Schroder et al., 2000; Kalsi et al., 2004).
As early as 1980 the idea was proposed that K^+ could be redistributed by Kir channels along the membrane of a single cell, or a network of cells connected via gap junctions from a region with high [K^+] to a region with lower [K^+] (Orkand et al., 1980). This spatial buffering hypothesis was strengthened by the finding that Kir4.1 expression varies within a given brain region. In fact, Kir4.1 shows distinct subcellular localisation, consistent with a specific role in transport of K^+ by glia between neurons and blood vessels. Kir4.1 is predominantly localised at distant astrocyte processes surrounding synapses or blood vessels (Nagelhus et al., 1999, Higashi et al., 2001). This polar distribution of Kir4.1 strengthens the hypothesis that potassium released by active neurons, is taken up into the astroglial syncytium and then distributed to blood vessels at the glia-vascular interface, as illustrated in figure 3.

K^+ buffering is a fundamental property of normal brain functioning. All neuronal activity, strictly every single action potential, leads to release of K^+ from neuronal tissue into the ECS. Without a rapid and effective buffering mechanism, any increase in extracellular K^+ concentration could compromise neuronal firing and normal brain functioning.
Lack of Kir4.1 channels will result in depolarization of glia, increased excitability and propensity to seizures (Kofuji et al., 2000; Djukic et al., 2007), as shown in Kir4.1 -/-

mice. Interestingly, not only is K^+ buffering markedly impaired in these mice (Chever et al., 2010; Haj-Yasein et al., in press), but also uptake of the excitatory neurotransmitter glutamate (Djukic B, 2007). Glutamate is most effectively taken up by astrocytes at negative resting potentials. Hence, impaired expression of Kir4.1 leads to reduced glutamate uptake and increased propensity to seizures.

Even subtle changes in Kir4.1 function could result in defective regulation of $[K^+]$ and thus present an attractive mechanistic hypothesis for an association between genetic variation and seizure susceptibility. Recent genetic studies have indicated an association between missense variations in the gene encoding Kir4.1, *KCNJ10*, and seizure susceptibility in both mice and humans (Buono et al., 2004; Ferraro et al., 2004). Bouno et al. detected a non-synonymous SNP (rs1130183) in the human *KCNJ10* gene (Arg271Cys) that was associated with seizure resistance in groups of patients with either focal or generalised epilepsy (Buono et al., 2004). In addition, the recent identification of a syndrome characterised by epilepsy caused by mutations in *KCNJ10*, emphasises the importance of Kir4.1 in cerebral $[K^+]$ regulation and the potential for *KCNJ10* as a seizure-susceptibility gene (Bockenhauer et al., 2009; Scholl et al. 2009).

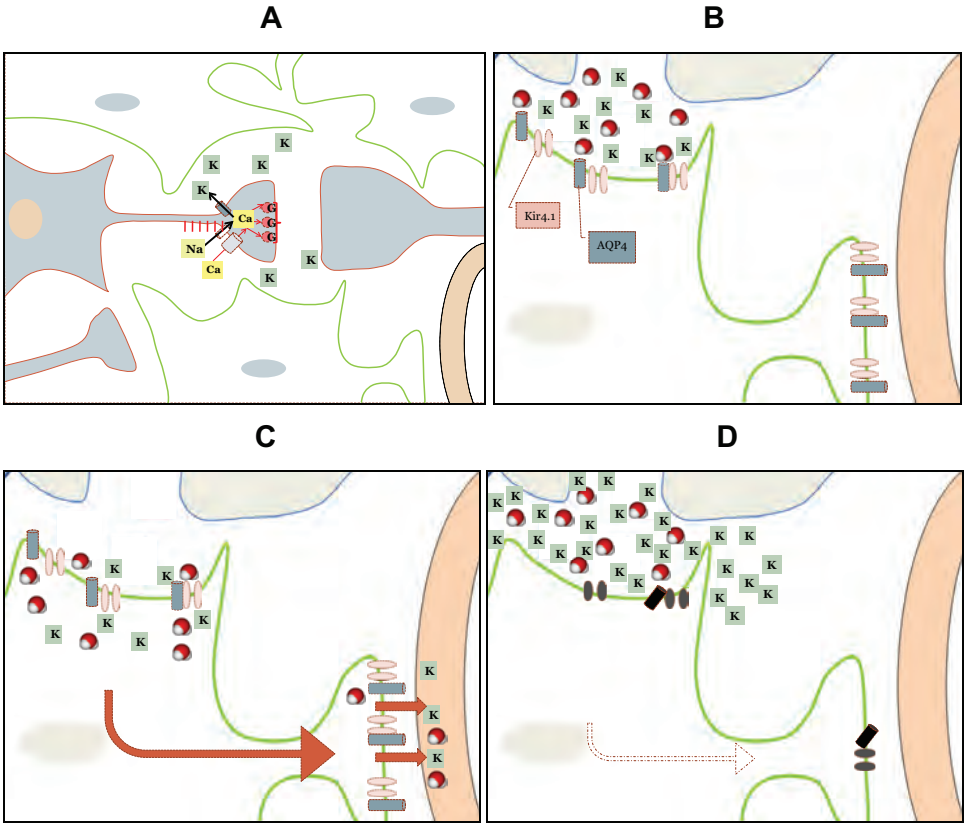


Figure 3: Presumed mechanism of potassium spatial buffering by the astrocyte membrane channel Kir4.1.

A: Potassium efflux from synaptic terminal during neuronal activity; **B:** Uptake of potassium by astrocyte Kir4.1 channels and possibly a concomitant water influx via water channel AQP4 to dissipate imbalance of ion concentration; **C:** Potassium is spatially redistributed by buffer currents and preferentially released to the perivascular space due to clustering of Kir4.1 channels in astrocyte endfeet. **D:** Defective Kir4.1 and/or AQP4 leads to increased extracellular potassium concentration and depolarisation of neuronal tissue. Perturbed K^+ clearance may play a role in epileptogenesis.

1.3.4 Roles of the water channel aquaporin-4

Water homeostasis is a fundamental property of all living organisms. Thus, the mechanisms of how water passes cell membranes are of fundamental interest. For about 200 years, diffusion, first recognised by the French physiologist René Joachim Henri Dutrochet (1776-1847), was believed (albeit debated) to be the basic way by which water passed through the lipid bilayer of plasma membranes. This view was revolutionised by the detection of specific transmembrane water channels, later named aquaporins, in 1991 (Preston and Agre, 1991). For this discovery, Peter Agre was awarded the Nobel Prize in Chemistry in 2003. As with diffusion, the transport of water through aquaporins is energy-independent and driven by an osmotic gradient across the plasma membrane. However, the velocity of water flux through aquaporins greatly exceeds that of diffusion. To date, 13 mammalian members of the aquaporin family have been characterised, with localisation in different organs. In the CNS, 3 water channels have been detected, AQP1, AQP4, and AQP9. Aquaporin 1 is expressed in the epithelium of the choroid plexus and is most probably relevant for cerebrospinal fluid (CSF) secretion (Nielsen et al., 1993). Aquaporin 9 is permeable not only to water, but also to a range of other molecules. AQP9 is expressed in tanocytes of the third ventricle ependyma, and also in astrocytes and neurons. AQP9 may be involved in brain energy metabolism, but its exact role is still unclear (Badaut et al., 2004). The role of aquaporin-4 (AQP4) has been studied in most detail and is also of principal interest in the context of this thesis.

AQP4 mRNA was originally detected in brain by Jung et al. in 1994 (Jung et al., 1994), and the protein was later shown to be expressed in astrocytes, with a polar distribution in perivascular endfeet and in the glia limitans towards the pial surface (Nielsen et al. 1997; Amiry-Moghaddam, 2004), figure 4. This distribution, with the highest density towards the brain-liquid interfaces, implies an important role in water homeostasis in the brain.

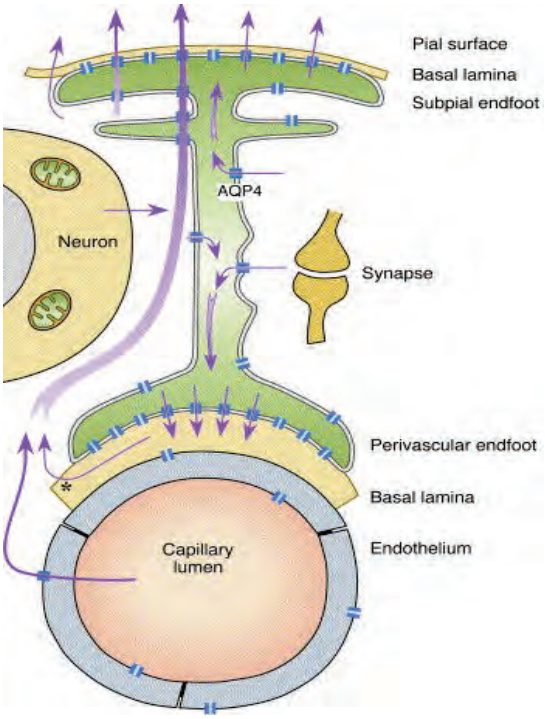


Figure 4: Distribution of AQP4 in the astrocyte membrane in the brain.
AQP4 is strongly enhanced in the astrocyte membrane towards the basal lamina surrounding blood vessels and towards the pial surface. Arrows indicate hypothetic water flux via AQP4 (Amiry-Moghaddam, 2004).

AQP4 regulates brain oedema formation and resolution in a range of neurological conditions, such as stroke, glioblastoma multiforme, brain abscesses, and hydrocephalus (Manley et al., 2000; Amiry-Moghaddam et al., 2003; Bloch et al., 2005; Papadopoulos and Verkman, 2005). AQP4 has also been implicated in K+ clearance, neuronal excitability and epilepsy (Eid et al., 2005; Binder et al., 2006), but its precise roles in brain are still elusive. It has been shown that the ECS shrinks significantly during neuronal depolarisarion (Dietzel et al., 1982). Shrinkage of the ECS could occur via AQP4 mediated water transport into perisynaptic astrocyte processes. The observation that the ECS at the same time increases in the neuropil distant to active synapses (Niermann et al., 2001), led to the theory that perisynaptic uptake of water is followed by efflux of water at a distant astrocyte membrane compartment.

1.3.5 Functional association between Kir4.1 and AQP4

AQP4 and Kir4.1 have entirely distinct functions, in as much as they selectively transport water and K^+, respectively. However, based on the close spatial relationship between these proteins in glial membrane domains, it was suggested that there is an obligatory coupling between water transport and K^+ clearance in the CNS (Nagelhus et al., 1999). In support of this hypothesis, high frequency stimulation of thalamic afferents to layer IV in acute cortical slices induces water fluxes to the superficial cortical layers, along with K^+, as recorded by K^+-sensitive microelectrodes (Niermann et al., 2001). Testifying to the interdependence of these transport processes, deletion of AQP4 (Binder et al., 2006) or elimination of the endfoot pool of this water channel (Amiry-Moghaddam et al., 2003) delays K^+ clearance and increases the severity of seizures The functional relationship between AQP4 and Kir4.1 is, however, debated. Verkman's group failed to show altered Kir4.1 channel function in AQP4 deficient mice (Zhang and Verkman, 2008). On the contrary, Soe et al reported that Kir4.1 channels are sensitive to cell volume changes and thus dependent on AQP4 (Soe et al., 2009). Moreover, a functional and molecular interaction between AQP4 and the Na+/K+ATPase (Illarionova et al., 2010) could also underly the effects of AQP4 deletion/mislocalization on extracellular K+ kinetics. Recently, AQP4 deletion was shown to delay recovery of extracellular K+ in the hippocampal stratum pyramidale, whereas K+ flux to stratum radiatum was enhanced (Strohschein et al., 2011). Impaired K+ uptake by the Na+/K+ATPase and facilitated K+ buffering by increased gap junctional coupling, were suggested to account for these opposite effects on K+ dynamics.

Studies in humans support the idea that certain forms of epilepsy are linked to perturbations in water and K^+ homeostasis. Most notably, the K^+ buffering capacity in MTLE-HS hippocampi is reduced in comparison with non-MTLE-HS hippocampi (Bordey and Sontheimer, 1998). This change is most pronounced in the CA1 region, where patch clamp experiments have demonstrated impaired uptake of K^+ into astrocytes through inwardly rectifying K^+ channels (Hinterkeuser et al., 2000). In addition to altered potassium ion kinetics, there is strong evidence that water accumulates in sclerotic hippocampi, as shown in MRI and in diffusion-weighted imaging of patients with MTLE-HS (Hugg et al., 1999). These functional changes mesh nicely with the finding that patients with MTLE-HS display a partial loss of AQP4 from astrocyte endfoot membranes (Eid et al., 2005).

1.3.6 The dystrophin-associated protein complex (DAPC) - an anchoring site for Kir4.1 and AQP4?

Dystrophin is a rod-shaped cytoplasmic protein and a vital part of a large protein complex, the dystrophin-associated protein complex (DAPC). The DAPC includes dystrophin and a number of dystrophin-associated proteins, which together connect the cytoskeleton to the surrounding extracellular matrix through the cell membrane. The dystrophin gene, with locus *Xp21*, has a length of 2.4 megabases and is the largest gene of the human genome. Mutations in the dystrophin gene are associated with neurological diseases, such as Duchenne and Becker muscular dystrophies. The dystrophin gene transcribes various isoforms (i.e., proteins of varying lengths containing different segments of the basic dystrophin sequence, which are encoded by different mRNAs, generated mainly by unique, tissue-specific promoters, but also by alternative splicing or use of different polyA-addition signals). The full length isoform of dystrophin is Dp427 (427 kDa), which is found mainly in skeletal muscle. In the brain, the predominant isoform is Dp71, which is expressed in astrocytes (Lederfein et al., 1992).

Altered DAPC has recently been implicated in animal models of status epilepticus (SE), indicating that dysfunction of dystrophin induced by SE may result in endothelial and astroglial damage, with breakdown of the blood-brain barrier (BBB) and increased vascular permeability as part of the pathogenesis of epilepsy (Sheen et al., 2010). Moreover, epilepsy is reported to occur at a higher incidence in patients with Duchenne and Becker muscular dystrophies (Goodwin et al., 1997; Tsao et al., 2006). This could indicate that alterations in the DAPC may lead to an increased propensity to seizures.

Nevertheless, little is known about the role of the DAPC/dystrophin-glycoprotein complex (DGC) in the human CNS physiology and pathophysiology, or the epileptic brain. Altered anchoring via DAPC may cause lack of Kir4.1 and also other important channels, such as the brain water channel, aquaporin-4 (AQP4), and thus critical for epilepsy development.

There is accumulating evidence that both AQP4 and Kir4.1 are anchored to the DAPC, supporting their co-active function in water and potassium homeostasis in the brain. This theory was originally generated by results obtained from double immunogold labelling in retinal Müller cell membranes that revealed co-enrichment of

Kir4.1 and AQP4 in vitreal and perivascular endfeet membranes (Nagelhus et al, 1999). Their close vicinity strengthens the theory that both channels are attached to the DAPC. Both channels are arranged in a polar distribution, with highest density in astrocyte perivascular endfeet and in astrocyte processes that form the glia limitans.
Further evidence for DAPC association with AQP4 comes from strains of mice that lack dystrophin (mdx mice), which display strongly reduced perivascular AQP4 expression (Liu et al., 1999; Nico et al. 2003).

Kir4.1 is localised in glial cells by its association with the DAPC through a PDZ domain-mediated interaction, as shown in immunoprecipitation experiments in mouse brain as well as in cultured cortical astrocytes (Connors et al., 2004), see figure 4.
Further support for a functional interaction between AQP4 and Kir4.1 has been obtained from studies on alpha-syntrophin -/- mice, which display serious mislocation of AQP4 and also delayed K^+ clearance, despite unchanged expression of Kir4.1 (Amiry-Mogghadam et al., 2003). Furthermore, it has been shown that KO of dystrophin 71, another member of the DAPC, results in decreased AQP4 and Kir4.1 expression (Dalloz et al., 2003).
Taken together, these data suggest an anatomical and functional entity between AQP4 and Kir4.1.

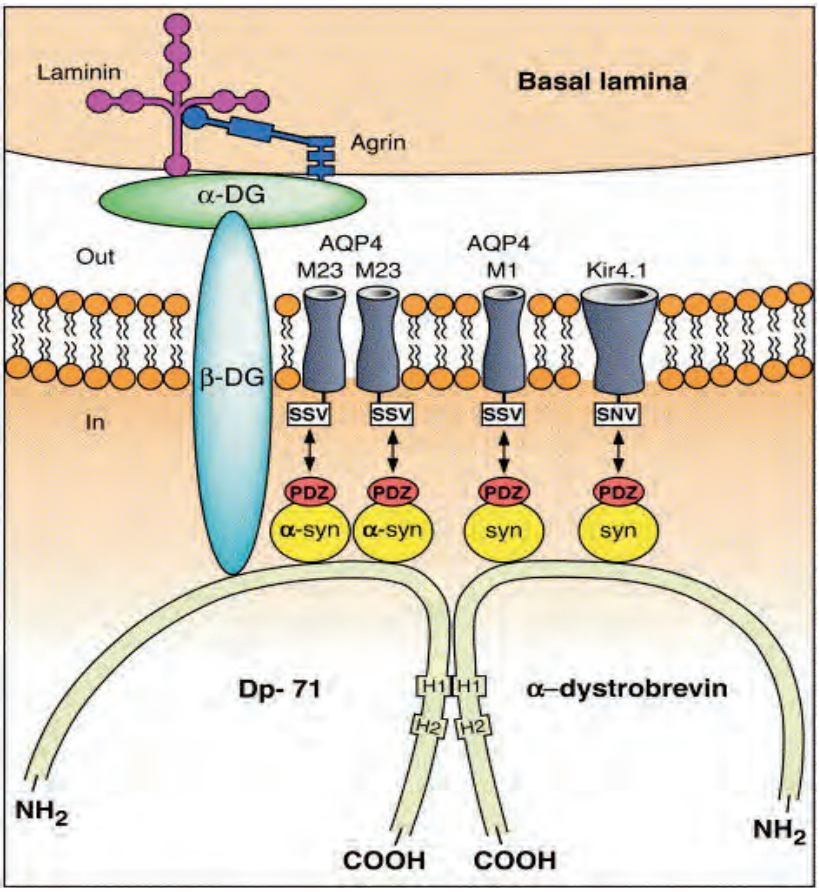


Figure 5: Diagram showing the presumed molecular basis for anchoring of AQP4 and Kir4.1 in the perivascular and subpial astrocyte membrane.

The dystrophin complex is anchored to the basal lamina via laminin and agrin and is presumed to bind AQP4 and Kir4.1 by way of alpha-syntrophin or other syntrophins. Other molecules involved in anchoring are the PDZ binding domain and beta-dystroglycan. H1 indicates the coiled-coil motif interaction between Dp71 (the major dystrophin isoform in brain) and alpha-dystrobrevin (Amiry-Moghaddam et al., 2004).

2. Material and methods

2.1 Phenotype studies on a Norwegian TLE population

2.1.1 Study population and data assembly

The original collection of patient data was carried out between 2000 and 2004 at seven tertiary Norwegian Medical Centres in a cooperative project (GenEpa), and was initially supported by GlaxoSmithKline. Hospitals involved in data assembly were the former Rikshospitalet University Hospital and the former Ullevål University Hospital, since merged to Oslo University Hospital; the former National Centre for Epilepsy, Sandvika; St Olav University Hospital, Trondheim; Haukeland University Hospital, Bergen; and Akershus University Hospital, Lørenskog. The aim of the initiative was to accrue a large dataset of Caucasian epilepsy cases and ethnically-matched controls, and to associate DNA sequence allelic variations in candidate genes with epilepsy phenotypes. After discontinuation of the GenEpA initiative an agreement was made between GSK and the principal investigator of the Norwegian GenEpa group, Professor Leif Gjerstad, which enabled handling of the GenEpA raw data within the framework of this thesis.

In total, 218 patients with TLE (according to the ILAE criteria, classification 1989) were included in the study. Inclusion criteria for all individuals were: age > 18 years; Caucasian race, with at least three of four grandparents of Scandinavian origin. The controls had no known familial relation to the TLE patients (typically spouse or partner). Standardised evaluation forms were used for all TLE patients and controls.MRI (typically 1 or 1.5 T, with sagittal and axial T1, axial and coronal T2, and Fluid-Attenuated Inversion Recovery (FLAIR) was performed in all 218 patients in order to differentiate cases with hippocampal sclerosis (MTLE-HS) from the remainder of the TLE patients (including patients with neocortical TLE and MTLE patients without HS). We identified 56 patients with MTLE-HS and 162 cases with other TLE. 102 patients had a history of febrile seizures, while 105 did not report any febrile convulsions in childhood. For 7 patients febrile seizure status was inconclusive.

2.1.2 Explorative study variables – phenotype factors

Patient groups were compared with respect to seizure semiology, age at epilepsy onset, appearance of FS in the patients and in first-grade family members, comorbidity with psychiatric and somatic diseases, and years of formal education. Demographic data (including ethnic background of 4 grandparents) were evaluated in all patients and controls. General medical history, including former and present antiepileptic medication, with duration of use and efficacy, were assembled. Diagnostic criteria for TLE, as described by the ILAE, were adhered to when establishing the diagnosis (epilepsy onset, seizure semiology, seizure type and frequency, etc., see also http://www.ILAE.org). Patient histories of febrile seizures were obtained from medical records and anamnesis. Family histories of epilepsy and/or febrile seizures were collated. Neurological examinations were performed on all patients. Data from EEG, performed at any time, and MRI, performed within 5 years of study enrolment, were collected. Within both case and control subjects, additional phenotypic factors were measured, such as height, weight, and waist circumference. A short questionnaire on general medical history, mood disorders, and educational status was completed for all participants.

2.1.3 Statistical analysis of phenotype data

The two clinical/ epidemiological investigations performed as part of this thesis, were studies on different subgroups of patients in a mixed TLE population. In the broadest sense, both studies could be classified as pilot studies. To our knowledge, few, if any, studies on mixed cohorts of operated and non-operated TLE patients have been previously performed. In the first study (paper I), data were described by proportions and medians with ranges. Crude associations between variables were investigated using chi-square tests or Fisher's exact tests (when number of observations was too small for one or both variables). Due to the "pilot character" of the study, we did not correct for multiple testing.

In our second study, we tested whether a certain group of patients, TLE-FS, could be segregated from other TLEs. This is the first study that has systematically evaluated this patient group. This study should also be regarded as a pilot study, and therefore we did not adjust for multiple testing. Categorical variables were described by proportions and continuous variables by medians and ranges. Associations between variables were studied using chi-square tests or Fisher's exact tests. Crude

associations between the two patient subgroups and age of epilepsy onset were assessed using the Cochran–Armitage test for trend. To correct for possible confounding, caused by overrepresentation of MTLE-HS in TLE-FS, multiple logistic regression models were adjusted for MTLE-HS and the results expressed as odds ratios (OR) with 95 % confidence intervals (CI). P-values < 0.05 were considered statistically significant. Given the relatively large sample size, we were able to fit multivariate models and adjust for possible confounders in addition to simple univariate analyses (chi-square tests). However, most of our results are highly statistically significant even when compared at a stricter significance level of 1 % (i.e., $p < 0.01$).

2.2 Genetic association studies in a Norwegian TLE population

2.2.1 Study population and data assembly

The study population and data assembly for genetic studies are identical to those for the phenotype studies, as described in section 2.1.1.

2.2.2 Explorative study variables - genotype factors

Allelic sequence variations in candidate genes were investigated by genetic association studies, as summarised in table 2.

Table 2: Candidate genes chosen for genetic association studies

Candidate gene	Gene product	Locus	Length in bp	SNP detection
AQP4	Water channel aquaporin-4	18q11.2-q12.1	13,706	Comprehensive search for DNA variation was conducted by PCR. Resequencing of the 22 overlapping PCR amplicons, covering the whole AQP4 gene, revealed 51 putative SNPs.
KCNJ10	Inwardly rectifying potassium channel, Kir4.1	1q23.2	32,854	43 SNPs from *KCNJ10* were chosen from HapMap (www.hapmap.org) and dbSNP (www.ncbi.nlm.nih.gov/SNP)
MMP-9	Extracellular enzyme matrix metalloproteinase-9	20q11.2-q13.1	7,536	40 SNP from the *MMP-9* gene were used in the assay design. The SNPs were chosen from HapMap (www.hapmap.org) and dbSNP (www.ncbi.nlm.nih.gov/SNP)

2.2.3 Case-control constellations tested for association

The following case-control constellations were tested in all association studies performed in this thesis:

a) Temporal lobe epilepsy (TLE) versus healthy control subjects
b) Mesial temporal lobe epilepsy with hippocampal sclerosis (MTLE-HS) versus healthy control subjects
c) MTLE-HS versus other TLE (without MTLE-HS)
d) TLE with a history of febrile seizures versus healthy control subjects
e) TLE with a history of febrile seizures versus TLE without a history of febrile seizures

2.2.4 Validation and characterisation of SNPs

SNP genotyping on all cases and controls was performed using the MassARRAY system from Sequenom, www.sequenom.com (San Diego, USA). All SNPs with a known allele frequency in Caucasian populations were included in the initial primer design. Genotypes were assigned in real-time (Tang et al., 1999) by using the MassARRAY SpectroTYPER RT v3.4 software (Sequenom) based on the mass peaks present. All results were manually inspected, using the MassARRAY TyperAnalyzer v3.3 software (Sequenom).

2.2.5 Statistical data analyses

Analysis of single SNPs and haplotypes in cases versus control groups

HaploView 4.0 software package (Barrett et al., 2005) was used for defining haplotype blocks and for investigating possible associations between single SNPs and haplotypes within blocks. The criterion for block definition was that suggested by Gabriel et al., 2002. Both nominal p-values and p-values corrected for multiple testing were considered. The boundary used for indicating significance was set to 5 % for the single SNPs and haplotypes. Multiple corrections of the p-values were determined by repeating 10,000 random permutations of the case/control status.

Explorative data analysis

Prior to the modelling, allele combinations (AA, AB, BB) for each SNP were recoded into 0–1 dummy predictor variables (one dummy predictor for each allele present in the SNP).

Multivariate data analysis for SNP selection

Together with a categorical response variable (indicating the case/control status of each subject in the dataset), the dummy predictors were included in a multivariate variable selection approach based on Partial least squares (PLS) methodology (Westerhuis et al., 1998; Nocairi et al., 2005). The PLS methodology is useful for establishing low dimensional subspaces of predictors in regression and classification problems and prevents overfitted models in situations where the number of predictors islarge in comparison with the number of subjects investigated. In order to assure additional sparseness (avoiding models influenced by unimportant SNPs), we used the Powered PLS (Indahl, 2005) in combination with the jackknife approach (Martens and Martens, 2001). Full (leave-one-out) cross validation was used to estimate the appropriate subspace dimension, as well as the importance of the individual SNPs. In order to minimise the number of SNPs required for prediction of case/control status, the candidate SNPs found to be most significant by combining the Powered PLS and jackknife approaches were re-investigated by linear discriminant analysis (LDA) (Hastie et al., 2001). LDA is a well-established statistical approach for solving classification problems. A group centre (prototype subject) is estimated for each group (here we have considered two groups, subjects and controls). Classification of a subject is obtained by assigning it to the group corresponding to the closest group centre according to a metric that is estimated as common within the group's covariance structure.

Predictive modelling and significance

The precision (associative strength) of the LDA-model defining the appropriate covariance metric from the final set of selected SNPs was measured as the percentage of correctly classified individuals computed by full cross validation. The boundary used for indicating model significance was set to 5 % (corrected for multiple testing of models). The significance numbers were estimated by 10,000 random permutations of the case/control status.

2.2.6 Probing non-coding SNPs for potential function

It is generally hypothesised that many disease-associated sequence variants in non-coding regions could influence the phenotype by altering gene regulation. In this respect, it is common to use evolutionary conservation as a guide for indicating non-coding regions with potential biological function (McCauley et al., 2007). Thus, for TLE-associated non-coding SNPs, we manually inspected the level of sequence conservation at their genomic locations using multiz28way alignments provided by the UCSC genome browser (Bejerano et al., 2005; Karolchik et al., 2008). Specifically, we looked for short (minimum 5nt) and conserved [all bases identical at the orthologous sites in monkey (rheMac2), mouse (mm8), dog (canFam2), and opossum (monDom5)] motifs that covered the SNP position. In addition to sequence conservation, we analysed whether any of the TLE-associated SNPs overlapped with known transcriptional enhancers (a genomic data track, provided by the UCSC genome browser).

2.2.7 Critical consideration of patient selection, data assessment, and interpretation

Phenotype definition problems and selection bias

Phenotype definition is a principal challenge in complex diseases like the epilepsies, and should always be an important concern in the early planning and design of any genetic association study. The specificity of the phenotype should be as high as possible. However, this task seems to pose particular difficulties, especially in the epilepsies, due to the sometimes subtle clinical presentation. The common clinical feature in epilepsies is the *seizure*, a mostly transient event, often never observed by the physician, and sometimes not observable at all. In these cases, diagnosis has to be established on other, indirect features, such as age at onset, seizure frequency, comorbidity, EEG findings, MRI results etc. From the opposite perspective, the same underlying disease entity may present a large variety of different types of seizures; the seizure itself usually does not always provide any further information about the aetiology and pathogenesis of the disease.

Another major issue for most genetic association studies is recruitment of a large number of subjects, which often requires collaboration between centres from different

geographical regions. This may lead to population stratification, due to mixing of genetically disparate subjects.
Another problem may arise from dissimilar routines in diagnostics and in data ascertainment.

In the studies described in this thesis, patients were recruited from seven different Norwegian hospitals, which may have affected phenotype specificity and could have introduced heterogeneity into the data. In this respect, it may be advantageous that most centres involved in subject selection are classified as tertiary centres, which implies a high level of competence in reaching a diagnosis and in phenotype selection. However, patients attending those facilities are often pre-selected, as they usually represent the most medicament-refractory and severe cases.
Every subject included in the studies described, had to be diagnosed according to highly rigid standards that were applied to ensure consistency and narrowness in phenotype definition. Cases in our study were chosen as specified in the 1989 ILAE classification (Proposal for revised classification of epilepsies and epileptic syndromes, 1989).

Population stratification and genetic heterogeneity

Population stratification

Population stratification is defined as the presence of a systematic difference in allele frequencies between sub-populations within a population due to different ancestry.
One major pitfall of genetic association studies is that population descriptions are sometimes vague. When exploring a set of genetic association studies, a large variety of population descriptions can be found. These range from use of racial group designations, to national labels, to geographic descriptors. Unclear assignment as non-random group sampling provides further confusion. These methodological failures often lead to population stratification, and result in replication attempts being almost impossible. Thus, there has been increasing focus on the use of a precise population description in genetic association studies (Kaplan et al., 2003; Fullerton et al., 2010).
Populations used in European association studies are often classified as “Caucasian”. In its original sense the term Caucasian (Caucasoid, Europoid) refers to the general physical type of populations from Europe, North Africa, and large parts of

Asia. In common use, especially in American English, the term is usually restricted to Europeans and other light-skinned populations within and around Europe, and may be considered equivalent to the varying definitions of white people.
Inconsequent use of terminology contributes to population stratification and lack of replicability in genetic association studies. Being aware of this issue, the population in our studies met stringent definition criteria, as described in the Methods section.

Genetic heterogeneity

Genetic heterogeneity is defined as the phenomenon that a single phenotype may be caused by any one of a multiple number of alleles or non-allelic (locus) mutations. One of the major difficulties in complex disease is the potential for genetic heterogeneity and differential interaction with a plethora of modifying genes. This issue is particularly serious for case - control designs in genetic association studies. It is the rule, rather than the exception, that datasets contain assortments of different aetiologies. Restricting ascertainment to a single ethnic group facilitates a reduction in this issue.
Even our own TLE dataset is not free from heterogeneity. TLE, by definition, contains a number of different aetiologies. For that reason, phenotype studies were conducted in an attempt to segregate particular subgroups of the TLE (papers I and IV). We found that the phenotype of TLE patients with hippocampal sclerosis (MTLE-HS) and the phenotype of TLE patients who had experienced febrile seizures in childhood (TLE-FS) could be segregated from other TLE, indicative of the existence of a specific aetiopathology. In our genetic studies, we looked specifically at genetic associations in these subgroups. Consequently, our phenotype study also served as a quality control for our genetic association studies.

Interpretation problems of genetic association studies

Despite a growing consensus that complex diseases, such as TLE, probably rely on a convoluted interplay between several, if not many, different genes, the idea still persists that diseases are usually caused by, at most, *one* single gene. This single gene is believed to be mainly responsible for the development of the disease, while others, so called modifier genes, may regulate/alter its expression and hence lead to penetrance variations (Greenberg et al., 2011).

The rationale for this gross oversimplification may be the fact that the analysis of such a complicated genetic interplay poses extreme challenges to our human conceptual skills, as well as data processing and interpretation. In multiple gene association studies or GWAS, the quantity of data generated is of such an immense dimension that difficulties arise even for single gene interpretation. In our association study, we established a multivariate analysis to explore specifically the possibility of allele combinations predicting disease. Since our study dealt with only 2 genes, the approach is rather simple, but is, nevertheless, more advanced than a simple single SNP association analysis. Tools for calculation and interpretation of gene-gene interactions in multiple gene association studies or GWAS are necessary to find common denominators of complex diseases like TLE.

2.2.8 Regulatory and Ethical Considerations

Handling of clinical patient data and blood/DNA was conducted in accordance with all applicable regulations, including, where relevant, the Declaration of Helsinki, June 1964, as modified by the 48th World Medical Association, Republic of South Africa, October 1996.

Ethics Committee

The protocol was reviewed and approved by the regional ethical committee (REC) before patient recruitment.

The REC reviewed and approved informed consent forms (ICF) and any other written information provided to the patient prior to any enrolment of patients.

Informed Consent

The Study Physician or his/her designee informed the subjects of all aspects pertaining to the subject's participation in the study.

The process for obtaining informed consent was in accordance with all applicable REC and regulatory requirements. The investigator, or his/her designee, and the subject both had to sign and date the ICF before the subject could participate in the study.

Data Security

Access to the data at the study site, DNA extraction laboratory, DNA Screening Centre, and the National Hospital of Oslo Norway was strictly controlled. Data containing the personal identification of the patients were kept in a separate file at the study site. The personal patient information was not transmitted to the extraction laboratory, the screening centre, or to any other third party.

2.3 Immunohistochemical studies on human hippcampal slices

2.3.1 Human subjects and definition issues

Patients with medication-refractory TLE were selected for surgery after phased clinical and electrophysiological assessment at Yale New Haven Hospital. Yale New Haven Hospital has a long tradition in evaluating epilepsy patients, and the Departments of Laboratory Medicine and Neurosurgery at Yale University School of Medicine embrace one of the world's largest brain banks for histopathological and molecular biological analyses of tissue derived from epilepsy patients. Patients selected for surgery underwent anteromedial temporal lobectomy, including resection of the hippocampus, according to standard procedures (Spencer and Spencer, 1991). Tissue used in this study was obtained after informed consent and with approval of the institutional human investigations' committee at Yale University. Hippocampal slices from TLE patients with hippocampal sclerosis (MTLE) and from those without sclerosis (non-MTLE) were randomly selected for this study. Random selection in this context means that the patients were not chosen on the basis of any clinical characteristics, but only on the basis of standard histology, showing either hippocampal sclerosis or not. Hippocampal sclerosis is defined by neuronal loss in distinct subfields of the hippocampus formation, mainly CA1 and the hilus, with relative sparing of CA2, in addition to astrocyte proliferation, granular cell dispersion, et ceteral. A somewhat critical point, which is often neglected, is the fact that tissue obtained from patients without hippocampal sclerosis is commonly described as "healthy" (non-sclerotic) control tissue. In fact, this is not absolutely correct. This control group may represent a mixed population of patients with different pathological features; some may present with mesial temporal mass lesions, while others may not show any obvious histopathological alterations at all. The only feature shared by this control population is seizure focus in the mesial temporal lobe. As the general research society usually does not distinguish between these entities, some authors have formulated nomenclatures to discriminate between them: The Yale group usually distinguishes between MaMTLE (mesial temporal lobe epilepsy on the basis of mass lesion) and PMTLE (pseudo-MTLE, without any obvious pathological changes in the hippocampus). For practical reasons, differentiation between MTLE and non-MTLE predominates and was also used in our histopathological study.

However, strictly speaking, differentiation should also be between subgroups within non-MTLE, especially for quantitative analyses, in order to minimise confounders.

2.3.2 Preparation of hippocampal slices

Immediately after resection, the hippocampus was cut into 5 mm coronal slices. Slices were obtained from the mid-anterior portion of the hippocampus and were immersed (1 h) in a fixative containing 4 % formaldehyde and 15 % (vol/vol) saturated picric acid in 0.1 M phosphate buffer, pH 7.4 phosphate buffer (PB). They were then transferred to 5 % acrolein (Sigma Chemical Co, St. Louis, Mo) in PB (3 h). Coronal sections were cut on a Vibratome and stored in a cryoprotection solution (FD Neuro Technologies, Catonsville, Md) at -20°C until further processing for Nissl staining and immunohistochemistry.

2.3.3 Immunohistochemistry

Vibratome sections of 50 µm thickness were incubated free-floating in the respective antibody solutions and processed according to the avidin biotin peroxidase protocol (Hsu et al., 1981) using the Vectastain Elite Kit (Vector Laboratories, Burlingame, Calif.) with diaminobenzidine as chromogen. The immunostained sections were mounted on gelatin-coated glass slides for examination by light microscopy The same method was also used to confirm specificity of the Kir4.1 antibody in fixed mouse tissue from *cKir4.1*$^{-/-}$ and wild-type mice.

Rabbit affinity-purified polyclonal antibodies against Kir4.1 (#APC-035, Alomone Labs, Jerusalem, Israel; 0.8 µg/ml incubated 72 h at 4 °C), dystrophin (#ab15277, Abcam, Cambridge, UK; 1 µg/ml incubated 24 h at 20 °C), and α-syntrophin (Syn259 kindly provided by S.C. Froehner, University of Washington, Seattle (Peters et al., 1997); 6 µg/ml incubated 24 h at 20 °C) were used and mouse monoclonal antibodies against β-dystroglycan (#B-DG-CE, Novocastra, 0.1 µg/ml incubated 72 h at 4 °C).

2.3.4 Semi-quantitative analysis

Semi-quantitative analysis, using bright field microscopy, was used in order to determine differences in Kir4.1 astrocyte immunoreactivity between MTLE and non-MTLE hippocampal specimens. Representative areas throughout all layers in the respective hippocampal subregions and in the hilus were marked, and immunoreactive astrocytes were counted per area. Mann-Whitney U-tests were used for statistical comparisons and a P value of <0.05 was considered statistically significant.

Semi-quantitative analyses in light microscopy are challenging and prone to confounding factors. Areas of interest in the tissue sections should be marked by a person other than the investigator. The investigator should not know whether the probe belongs to the case (MTLE) or the control (non-MTLE) group. Although these principles were adhered to in our analysis, as the pathological changes in MTLE are relatively obvious, blinding is difficult to achieve. Moreover, enumerating objects of interest (astrocytes in the case of our study) depends on the alertness and experience of the investigator, and on the visual acuity of the investigator. Thus, different investigators may achieve different results, and the approach is, therefore, rather subjective.

It is important to keep in mind that data obtained from human hippocampus tissue are, in all likelihood, not representative for the general TLE population, as patients selected for operation embody a kind of “end point” of a long-standing epileptogenic course. The most characteristic features of the sclerotic hippocampus, which are neuronal cell loss and gliosis, are accompanied by a multitude of molecular changes in glia, including the changes reported in this thesis. However, the aspect which remains elusive is the timeline of the impact of these changes and whether they represent causes or consequences of the epileptogenic process.

3. Summary of results

3.1 Paper I

Heuser K, Taubøll E, Nagelhus EA, Cvancarova M, Ottersen OP, Gjerstad L.

Phenotypic characteristics of temporal lobe epilepsy: the impact of hippocampal sclerosis.

Acta Neurol Scand Suppl. 2009;(189):8-13

The aim of this introductory study was to explore phenotypic characteristics of patients with MTLE-HS and to compare them with other TLE patients who did not present with hippocampal sclerosis.

The study was established on the basis of a large database, including 218 Norwegian TLE patients, generated from standardised patient record forms which, in turn, had been derived from the GenEpA project (see Materials and Methods).

We identified phenotypic characteristics that distinguish MTLE-HS from other TLE, and thus reinforce the theory that MTLE-HS may constitute a unique entity. This study provided a basis for the subsequent genetic association studies on subgroups of TLE and also served as quality control regarding our study population.

3.2 Paper II

Heuser K, Nagelhus EA, Taubøll E, Indahl U, Berg PR, Lien S, Nakken S, Gjerstad L, Ottersen OP.

Variants of the genes encoding AQP4 and Kir4.1 are associated with subgroups of patients with temporal lobe epilepsy.

Epilepsy Res. 2010 Jan;88(1):55-64

This candidate gene study aimed to investigate whether variants of the genes encoding AQP4 and Kir4.1 are associated with TLE or subgroups of TLE. For that purpose, DNA from 218 Norwegian patients with TLE and 181 ethnically-matched, healthy controls were included and single nucleotide polymorphisms (SNPs) for *KCNJ10* (Kir4.1 gene) and *AQP4* were identified via HapMap and/or resequencing. For the TLE cohort as a whole, explorative multivariate analysis indicated a combination of several associated SNPs in *KCNJ10* and *AQP4* genes. However, the strongest association was found by single SNP and explorative multivariate analysis in the TLE-FS subgroup, with seven SNPs in *KCNJ10* and one SNP between *KCNJ10* and the neighbouring gene, *KCNJ9*.

We concluded that variants of the *KCNJ10/KCNJ9* and *AQP4* genes are likely to be associated with TLE, particularly TLE-FS.

3.3 Paper III

Heuser K, Hoddevik EH, Taubøll E, Gjerstad L, Indahl U, Kaczmarek L, Berg PR, Lien S, Nagelhus EA, Ottersen OP.

Temporal lobe epilepsy and matrix metalloproteinase-9: a tempting relation but negative genetic association.

Seizure. 2010 Jul;19(6):335-8.

Matrix metalloproteinase-9 (MMP-9), a proteinase that cleaves extracellular matrix molecules, has been proposed as having a critical role in aberrant synaptic formation in the hippocampi of patients with TLE.

This case-control study was designed to identify possible variants of the *MMP-9* gene associated with TLE. A genetic association analysis was conducted in which 218 Norwegian patients with TLE and 181 ethnically-matched controls were compared. We also studied associations within two subgroups of TLE-- MTLE-HS and TLE-FS. SNPs were selected from HapMap and dbSNP databases for the *MMP-9* gene on chromosome 20, and standard haplotype analysis and multivariate explorative analysis were used.

We found no statistically significant associations between the selected SNPs in the *MMP-9* gene with TLE, or subgroups thereof.

3.4 Paper IV

Heuser K, Cvancarova M, Gjerstad L, Taubøll E.

Is Temporal Lobe Epilepsy with childhood febrile seizures a distinctive entity? A comparative study.

Seizure. 2011 Mar;20(2):163-6

Sufficient evidence exists to conclude a genetic propensity for the occurrence of FS, and various studies confirm a link to TLE. These studies were reinforced by data from our own association study, outlined in paper II, which indicated genetic variants in TLE-FS. The aim of this paper was to test the hypothesis that TLE with childhood febrile seizures (TLE-FS) represents a distinctive subgroup among the TLE.
On this basis, the question arose whether TLE-FS displays a phenotype that can be distinguished from other TLE. To address this issue, we compared clinical and epidemiological features from 102 TLE-FS patients with those of 105 TLE patients without FS. We also conducted logistic regression analysis to adjust for possible confounders caused by overrepresentation of patients with MTLE-HS in the TLE-FS group. This was reasonable, as MTLE-HS had been identified as a distinguishable subgroup in paper I.
We identified several clinical features significantly associated with TLE-FS, and concluded that TLE-FS is a phenotype that can be delineated from other TLE.

3.5 Paper V

Heuser K, Eid T, Lauritzen F, Thoren AE, Vindedal GF, Taubøll E, Gjerstad L, Spencer DD, Ottersen OP, Nagelhus EA, de Lanerolle NC.

Loss of Kir4.1 potassium channels in hippocampus of patients with mesial temporal lobe epilepsy

Manuscript submitted

Malfunction or lack of the astrocyte potassium channel Kir4.1 is assumed to be associated with epilepsy. To investigate the distribution of Kir4.1, immunohistochemistry was used in hippocampus specimens from patients with refractory MTLE-HS and compared with equivalent specimens from patients with TLE without hippocampal sclerosis. Immunohistochemistry was also conducted in the same patient material on molecules presumed to be involved in astrocyte membrane anchoring of Kir4.1, such as dystrophin, alpha-1-syntrophin, and beta dystroglycan. We found that Kir4.1 immunoreactivity in astrocytes was significantly reduced in patients with hippocampal sclerosis, in comparison with patients without sclerosis. The loss of astrocytic Kir4.1 immunoreactivity was most pronounced perivascularly, and was restricted to gliotic areas. Loss of Kir4.1 expression was associated with loss of dystrophin and α-syntrophin, suggesting disruption of the dystrophin-associated protein complex. In contrast, differences between MTLE and non-MTLE were not observed for beta dystroglycan staining.

The changes identified in patients with hippocampal sclerosis are likely to interfere with K^+ homeostasis and may contribute to the epileptogenicity of the sclerotic hippocampus.

4. Discussion

4.1 Which tools should be chosen for unravelling complex diseases?

Complex diseases cover a broad spectrum of human health. Genes are likely to control disease risk, and interactions between genes, or between genes and the environment, may also play an important role. Advances in human genetics could assist in optimising management of complex diseases, help to assess risk factors, aid in disease prevention, and be used to assess prognosis. Genetic studies have proved highly successful at detecting simple Mendelian diseases, where single gene mutations have large effects, but progress has been slow when it comes to detection of genetic factors in complex diseases, including the large majority of idiopathic epilepsies.

Several genes have been discovered as being causative for rare epilepsy forms or related syndromes. However, to date, there is no consistent evidence that these genes contribute to a predisposition to the common epilepsies, such as TLE.

An almost dogmatic consideration over the past two decades has been the belief that epilepsies are highly likely to be diseases of *neuronal* ion channels and/or receptors, which led to the term “neuronal channelopathies”. This assumption has probably prejudiced the search for alternative target genes in epilepsy and also restricted, at least in part, additional scientific approaches.

However, the “neuronal channelopathy hypothesis” is currently under reconsideration, based on the fact that the search for causative neuronal ion channel genes proved unsuccessful for common epilepsies like TLE (Tan et al, 2004).

The primary intention of this thesis was to investigate whether alternative “non-neuronal” factors, more specifically the astrocyte targets AQP4 and Kir4.1 and the extracellular enzyme MMP9, could be involved in the development of TLE.

Phenotype-genotype studies are auspicious for unravelling the genetic basis of complex diseases, including epilepsy. But such studies require a clear delineation of phenotypes. Thus, one objective of this thesis was to determine whether subgroups of TLE exist that could be delineated on the basis of clinical and demographic variables. This facilitated a quality control regarding our Norwegian TLE population,

and also formed the basis for our genetic case-control studies. Two subgroups were identified, and their classification resulted in the production of papers I and IV. As elaborated in the introduction, there is ample evidence in the literature for MTLE-HS to be considered a distinctive syndrome. However, the case differs for the subgroup of TLE patients who experience febrile seizures in childhood (TLE-FS). Despite a few indications in the literature, we found no earlier systematic approach for identifying TLE-FS as a separable subgroup.

4.2 Does MTLE-HS represent a distinct disease entity?

Clinical aspects of TLE have been studied extensively. However, there is a lack of analyses of phenotypic differences between MTLE-HS and other TLE-patients in a mixed cohort of operated and non-operated patients. Studies based solely on surgically-treated patients do not include the whole spectrum of MTLE-HS patients, as they concentrate only upon the most severe and medically-resistant cases. These gaps in our knowledge were addressed by the complementary study described in paper I.

We found phenotypic differences between MTLE-HS and other TLEs with respect to seizure type and semiology, interictal depression, and age at epilepsy onset, as well as frequency and family history of febrile seizures. These findings suggest dissimilar biological backgrounds and support the existence of a specific aetiopathology in MTLE-HS.

In our material, 26 % (56 patients) were diagnosed with MTLE-HS, in contrast to 74 % (162) with other TLEs. At first glance, the number of MTLE-HS cases seems low. However, a plausible explanation for this may be found in our study design: Most literature referring to MTLE-HS frequencies are based on histological evaluation of patients that have been operated for the condition. These studies generally report a proportion of 50 % MTLE-HS cases. Close study of the literature, however, indicates a variation from 10 % (Goldsmith et al., 2002) to over 50 % (Semah et al., 1998). We believe that the wide range of incidence values is a reflection of the challenge of data ascertainment in epileptic disorders. Notably, deficiencies in patient reporting and in the diagnosis of seizures are known confounders, as are selection biases and classification problems (Sander et al., 1987).

4.3 Does TLE-FS represent a distinct disease entity?

Sufficient evidence exists to conclude that there is a genetic propensity for the occurrence of FS, and an array of studies confirm a link to TLE, as elaborated in the introduction. Although specific genetic associations have not been found for the vast majority of FS and TLE, there is a growing consensus of opinion that genetics does have a role in both conditions. As both TLE and FS encompass strong clinical features, the combination of both in an individual may be a predictor for a genetic basis itself, and it is therefore reasonable to search for susceptibility genes and also phenotypic characteristics at the intersection of both conditions.

The study conducted in paper IV was motivated by findings from our association study that indicated genetic variation among TLE patients who have had febrile seizures in childhood (TLE-FS) when compared with TLE without FS (Heuser et al., 2010) As a result of these findings we wanted to test the hypothesis that TLE-FS represents a further subgroup among the TLE. A literature search for supporting evidence identified a few other studies that indicated TLE-FS to be a unique entity, distinct from afebrile TLE (Umbricht et al., 1995; Abou-Khalil et al., 2007; Wieshmann UC et al., 2008). However, to the best of our knowledge, our study is the first systematic investigation of the phenotype of TLE-FS.

We identified TLE-FS as a phenotype that can be delineated from other TLE on the basis of clinical and demographic features. Thus, TLE-FS may be considered to constitute a distinctive entity. However, none of the clinical characteristics could be regarded as specific. Further research, including genetic, clinical and animal studies, is needed in order to reach a firm conclusion.

4.4 Alternative routes for epileptogenesis – might these occur via glial cells?

As elaborated in the introduction, there is now a wealth of evidence that demonstrates that glial cells play an important role in the aetiology and pathogenesis of brain diseases, including the epilepsies.

Normal brain function is critically dependent on efficient mechanisms for clearance of excess K^+ from the ECS (Kofuji and Newman, 2004). A build-up of extracellular K^+

makes neurons hyperexcitable and may rapidly translate into epileptic seizures. Electrophysiological data suggest that MTLE may be associated with deficient K^+ handling and have indicated possible perturbations of K^+ transport through the family of inwardly rectifying K^+ channels (Bordey and Sontheimer, 1998; Hinterkeuser et al., 2000).

Within the scope of this thesis, we have demonstrated that Kir4.1 is lost from perivascular endfeet in hippocampi of MTLE patients. This loss occurs specifically in sclerotic hippocampi and is most pronounced in area CA1 and the hilus (figure 6). Loss of Kir4.1 shows a precise spatial coupling to regions of neuronal loss and gliosis. Thus, vessels typically lose their Kir4.1 immunopositive sheath as soon as they enter a sclerotic region.

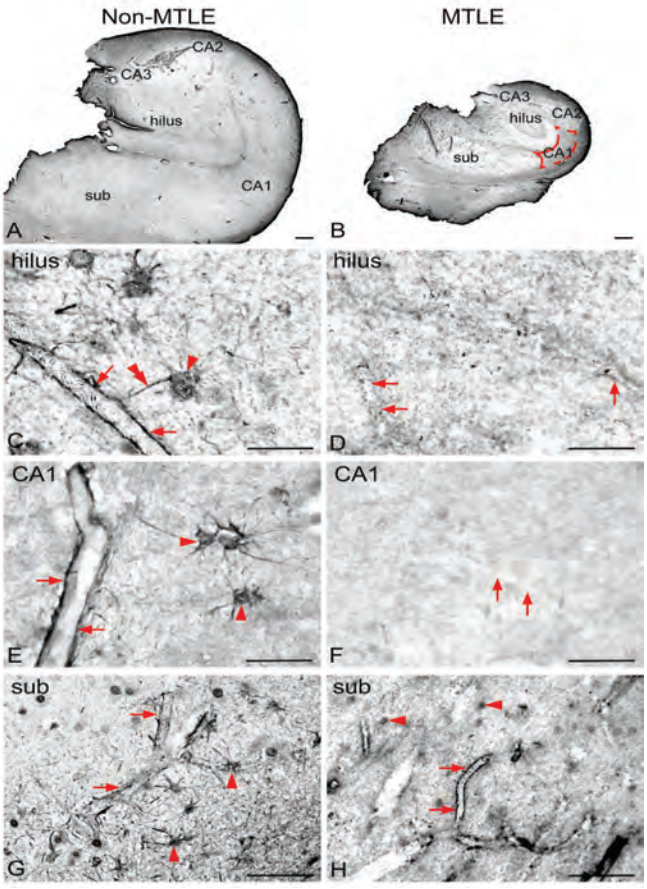


Figure 6: Distribution of Kir4.1 immunoreactivity in coronal sections of the hippocampus in patients with TLE.

Shown is Kir4.1 labelling in subfields of the hippocampal formation from patients without hippocampal sclerosis (non-MTLE) (A, C, E, G) and from patients with hippocampal sclerosis (MTLE) (B, D, F, H). In non-MTLE cases Kir4.1 immunoreactivity resided in astrocytic somata (arrowheads in C, E, G), processes (double arrowheads in C), and perivascular endfeet (arrows in C, E, G). Hippocampi from patients with MTLE showed substantial loss of astrocytic Kir4.1 immunoreactivity in areas with neuronal loss and gliosis (D, F). Thus, the sclerotic CA1 were almost devoid of Kir4.1 labelling (F). The hilus showed changes that were somewhat less extensive than those observed in CA1 (D). Loss of perivascular Kir4.1 immunoreactivity was associated with reduced labelling of astrocytes. The subiculum in MTLE (H) displayed a labelling pattern on a par with that observed in non-MTLE (G). The sclerotic area in CA1 in MTLE is marked with dashed line (B). Vertical panels (C, E, and G) and (D, F, and H) are high-magnification fields of the respective areas in A and B. Scale bars: A, B, 1 mm; B-F, 50 µm; G, H, 100 µm.

Our data also provide some insights into the mechanisms that underlie the observed loss of Kir4.1. Notably, the immunocytochemical analyses revealed that the changes in Kir4.1 distribution are associated with parallel changes in the distribution of dystrophin and α-syntrophin. The most salient explanation of this finding is that the loss of Kir4.1 is secondary to a disruption of the DAPC, of which α-syntrophin is known to be a member (Kofuji and Newman, 2004). If valid, this explanation implies that Kir4.1 is anchored to the DAPC, as has been reported previously (Waite et al., 2009). The changes incurred by disruption of local anchoring mechanisms may have been accentuated by alterations at the transcriptional or translational level, as indicated by the reduced number of Kir4.1 positive astrocytes in the gliotic areas.

Although the precise mechanism remains to be established, the loss of Kir4.1 from the hippocampus of MTLE patients is a finding of considerable interest, as this loss may be an important contributing factor to epileptogenesis. Our finding complements previous electrophysiological observations in MTLE hippocampi that have indicated deficiencies in K^+ homeostasis. Recent data from our own laboratory clearly show that deletion of Kir4.1 in mice delays K^+ clearance and interferes with K^+ spatial buffering (Haj-Yasein et al., in press). Mice with deletion of Kir4.1 succumb to severe seizures at an early age (Djukic et al., 2007). Taken together, the available data indicate that the loss of Kir4.1 could be a key step in the cascade of events that culminate in the development of chronic epilepsy.

4.5 Alterations in the dystrophin associated protein complex (DAPC) - associated with loss of Kir4.1?

The changes in Kir4.1 labelling illustrated were mimicked by changes in dystrophin labelling. Notably, the perivascular labelling for dystrophin was lost once the vessels entered the gliotic areas in CA1 or hilus. Vessels in the granule cell layer and subiculum were associated with strong dystrophin immunolabelling in MTLE as well as in non-MTLE hippocampi.

Labelling with the antibody to α-syntrophin produced a pattern that was strikingly similar to that found after application of the antibody to dystrophin, and this was true

for sections obtained from MTLE patients as well as for sections from non-MTLE patients.
Interestingly, labelling for beta-dystroglycan did not show any differences between MTLE and non-MTLE cases and no changes in the sclerotic hippocampal subfields.
Our finding that dystrophin and α-syntrophin are lost while β-dystroglycan persists is consistent with the idea that MTLE is associated with activation of an intracellular protease that cleaves dystrophin. It is interesting in this regard that excitotoxicity has been shown to induce activation of calpain, an enzyme with a known affinity for dystrophin (Araujo et al., 2010; Yoshida et al., 1992). Attesting to the clinical significance of Kir4.1, the human Kir4.1 gene (*KCNJ10*) is associated with epileptic disorders (Buono et al., 2004; Heuser et al., 2010). Moreover, it was recently reported that mutations in the *KCNJ10* gene give rise to a syndrome consisting of epilepsy, ataxia, sensorineural deafness, and tubulopathy (EAST/SeSAME syndrome) (Scholl et al 2009; Bockenhauer et al., 2009)

There is one basic limitation when investigating astrocyte targets that should be taken into consideration. Due to the fact that the interactions between neurons and glial cells are not fully understood, it is difficult to determine whether changes in glial cells in epileptic tissue are causes or consequences of the disease.

4.6 Why do genetic association studies fail?

Genetic association studies are regarded as powerful tools for discovering common variants in complex and common diseases. However, association studies have performed relatively poorly to date as has been widely described, including a cross-disciplinary review that showed consequent replication in not more than 6 out of 600 associations (Hirschhorn et al., 2002).
This rather sobering realisation has led to the implementation of stricter requirements for adequate methodological approaches in genetic association studies over recent years, including in the epilepsy field (Tan et al., 2004).
Medical journals have become more restrictive at publishing association studies that do not fulfil requirements such as clear population definitions, correction for population stratification, selection of appropriate control subjects (ethnically-matched, from the same geographical region etc.), sufficient sample size, and high biological

plausibility (the validity of the study has to be demonstrated in a meaningful scientific context) for the choice of the candidate gene.

In particular, it is currently almost impossible to publish negative association studies. This restrictiveness is not necessarily advantageous, as it bears the risk of publication bias. Another important weakness that may evolve from insisting on a substantial sample size is that rare idiopathic diseases, including rare epilepsy forms, may not be considered for investigation, even though such studies may provide valuable information that is of relevance to specific issues. Furthermore, studies with large sample sizes often require collaboration across geographical and thus "genetic borders", which may dilute phenotype specificity.

The latter is a major challenge for archetypical GWAS with large sample sizes, which have become increasingly common in recent years. Although GWAS are considered highly promising and successful (Hindorff et al., 2009), they are not completely sacred, and the theory that GWAS would solve all the problems encountered in single gene association studies has not been proved after all. Indeed, the enormous quantities of data that are generated by GWAS seem almost impossible to handle, and separating the few "true" signals from the extensive background noise is a serious challenge. Methodological technology has not kept pace with the flood of genotyping data; exploring gene-gene interactions (epistasis) involves a heavy computational burden, and things get even worse when environmental factors are added in to the equation. In fact, the abundance of data GWAS provides, may have confused our discrimination between true scientific and false discoveries. As GWAS seem to be in danger of becoming placeholders for all other approaches to detecting genetic risk factors for complex/common diseases, these difficulties should not be forgotten in our enthusiasm.

In my opinion, single gene or few-gene association studies still have a place in genetic research, as long as the appropriate criteria are maintained. These guidelines were, on the whole, intended to adhere to in the protocol for our association studies, as is reflected by the following: we aimed to use a phenotype that had been defined as clearly as possible; we had an *a priori* hypothesis and a strong biological plausibility for our candidate genes; population stratification was minimised by using only Norwegian subjects with Norwegian ancestors for both cases and controls. In addition, and to meet some of the challenges illustrated above, we conducted phenotype studies to achieve statistical support for the selection of TLE subgroups

used in our association analyses and used multivariate analysis in searching for disease-predicting allele combinations. Although we were unable to identify any polymorphisms of the human *MMP*-9 gene that were associated with TLE or the subgroups TLE-FS and MTLE-HS (Heuser et al., 2010), our search for associated variants of the *AQP4* and *KCNJ10* gene proved successful (Heuser et al., 2010).
However, our sample size was rather moderate, and bias caused by selection, self reporting, population stratification, and genetic heterogeneity may have resulted in type I errors in our Kir4.1/AQP-4 study. For similar reasons, the negative results of our MMP-9 association study may be a type II error.

Association studies are based on the theory that common, low penetrance genetic variants could cause, or be associated with, common diseases. Indeed, some studies have identified a large set of genetic variants that contribute to a great variety of traits and common diseases. However, disappointingly, both individual and cumulative effects are far too small to explain earlier estimates of heritability. Hence the "common variant – common disease" theory" for complex diseases has to be reconsidered.
As highlighted by Maher, 2008, the search for heritability factors may fail due to:
1) Limitations regarding the design of association studies, as outlined above.
2) There may be hundreds or thousands of common variants with low penetrance that invoke heritability. We lack sufficient modelling skills to assess their cumulative effect, and it is doubtful whether such a model would have any relevant consequences for clinical practice.
3) The DNA architecture contains multiple large stretches of DNA, ranging from thousands to millions of bases, which are deleted or duplicated. These segments, also termed *copy number variations (CNV)*, may account for genetic variability. As with other types of genetic variation, they probably also play a role in the heritability of diseases (Stefansson et al., 2008). Technology for detecting CNV is currently being tested by a consortium called the *CNV project* (Wellcome Trust Sanger Institute, Cambridge, UK; http://www.sanger.ac.uk/humgen/cnv).
4) Epistasis can be defined as the phenomenon of the interaction of genes. Effects of one gene can be modified by one or several other genes. Epistasis has been recognised as fundamentally important to understanding the functions of genetic pathways and evolutionary dynamics of complex genetic systems (Phillips P, 2008).

Little is known about these gene-gene interactions, and no technology exists to aid the search for effects caused by epistasis.

5) Epigenetics: the mechanisms of epigenetics are discussed in more detail in the subsequent section.

6) Another possible explanation for the unsuccessful search for inheritability in complex diseases may be simply the fact that our thinking is fundamentally wrong, and that other, currently unconsidered, factors are responsible or of greater importance.

4.7 What makes us what we are?

It is important to remember that *we are not our genes*. Our genome cannot fully determine everything that we are, think, do, look like, and neither can it be entirely blamed for our susceptibility to diseases. The functionality of our cells, our organism, and, in a broader term, our "body and mind", depends on more than on a helix of acidic base-paired nucleotides. But what else makes us what we are? At this point in the discussion it may be tempting to drift off into religious or philosophical considerations. Although this may be particularly interesting, I prefer to leave this question unanswered. To return to applied science, the rapidly progressing field of *epigenetics* cannot be omitted from the discussion. Although already defined in 1939 (that is, 14 years prior to the discovery of the molecular structure of the DNA) by the English biologist and geneticist Conrad Hal Waddington (1905-1975) as "the causal interactions between genes and their products, which bring the phenotype into being", this field is really just at the beginning of its scientific journey. This is attested by the over tenfold increase in the amount of publications on this topic within the past decade (Portela et al., 2010). Epigenetics refers to the dynamic chemical modifications that occur to our DNA, as well as its subsequent association with regulatory proteins (Esteller M, 2008). To describe even the best recognised epigenetic modifications, such as DNA methylation, histone modifications, and nucleosome positioning, would go far beyond the scope of this thesis. All human cells share the same genetic information, but brain cells definitely have other duties than cells of, for example, the intestinal mucosa. This means that not every gene can be active in every cell all the time.

As genetics (from Greek genetikos/ genesis = origin) can be considered as the origin of life, it can also be claimed to be the origin of all living organisms, of the individual, the human with all its different characteristics, including the diseased one. This latter consideration is the reason why disease classifications consistently fail, due to the challenging act of drawing a line between disease and non-disease, and, probably even more difficult, in separating disease entities from each other.

Genetic factors probably profile a continuum from healthy to diseased, and, in our context, from a “normal” brain, via a slightly excitable brain, to the hyperexcitable (epileptic) one. Moreover, environmental factors may cause disease under certain circumstances, such as, for example, a definite genetic predisposition.

Recent advances in molecular genetics have added important value to our understanding of complex diseases, including the epilepsies. The discovery of monogenetic epilepsies has contributed to our knowledge of underlying aetiopathogenetic mechanisms of different epilepsy forms. Genetic tests have become available for different specific autosomal dominant familial epilepsies, and thus influence our clinical diagnostic practices. There is good reason to believe that considerable progress will also be made for common epilepsies with a complex genetic background, such as TLE and FS.

However, to end with a quotation from Leonardo da Vinci: “All our knowledge has its origin in our perceptions”. Whether human perception or intelligence will ever be sufficient to uncover and understand the complex relationships between genes, gene products, external factors, and disease development currently remains unanswered.

5. Conclusion

- We conclude that it is possible to delineate phenotype subgroups among the TLE on the basis of demographic and clinical variables.

    - We confirmed that patients with MTLE-HS constitute a sufficient cluster of signs and symptoms to represent a distinctive syndromic entity.

    - We identified that TLE patients who suffered from early childhood febrile seizures (TLE-FS) represent a phenotype distinguishable from other TLE patients.

- We conclude that variants of the *KCNJ10* (Kir 4.1) gene and *AQP4* gene are associated with TLE, particularly the subgroup of TLE-FS, supporting the suggestion that TLE-FS may constitute a unique entity, and that perturbations in water and K^+ transport are involved in the aetiopathogenesis of TLE.

- We conclude that there is significant loss of the potassium channel, Kir4.1, in the sclerotic hippocampus of patients with MTLE-HS, and that this loss may contribute to epileptogenesis in MTLE.

- We hypothesise that deficient Kir4.1 in MTLE-HS is a consequence of altered anchoring via the dystrophin associated glycoprotein complex (DAPC).

6. Future studies

Within the period in which this thesis has been developed, we established an international network of research groups from both Europe and the USA. Parts of the research described in this thesis have, as noted, been conducted together with our collaborators at the Departments of Laboratory Medicine and Neurosurgery at Yale University School of Medicine, New Haven, CT, USA. Since then, this collaboration has been strengthened. In addition, we have established partnerships with groups in Bonn, Germany, Kuopio, Finland, and Utrecht, The Netherlands. Together with these European collaborators, we have formulated a framework for future projects, some of which are outlined below.

Risk factors for temporal lobe epilepsy; gene association studies of different temporal lobe epilepsy subtypes in large TLE cohorts

We plan to establish a set of association studies in well-defined subgroups of TLE, such as TLE-FS, MTLE-HS, drug-resistant TLE, and TLE patients with family members with TLE or other epilepsy types. These studies will focus on targets in brain glia. In order to increase statistical power and to exclude rare familial TLE subtypes, cohorts with large numbers of patients and matched-controls will be recruited from collaborating centres.

Receptor-mediated Ca^{2+} signalling and gliotransmitter release

Glial function is intimately regulated by cellular calcium signalling that underlies the specific form of "glial calcium excitability" (Nedergard and Verkhratsky, 2010). It has been shown that increased Ca^{2+} signalling in astrocytes results in paroxysmal depolarisation shifts (Tian et al., 2005), which characterises all focal epilepsies neurophysiologically, indicating a key role for astrocytes in seizure activity.

Recent, two-photon imaging data from our own laboratory, show that AQP4 facilitates glial Ca^{2+} signalling *in vivo* and in cortical slices (Thrane et al., 2011). This might, therefore, contribute to glial Ca^{2+} hyperactivity, and hence the observed deleterious effects in the affected brain tissue. Our hypothesis is that glial cells in patients with MTLE-HS express pathologically increased Ca^{2+} signalling, leading to elevated

glutamate release and epilepsy. If confirmed, this could pave the way towards completely new treatment strategies for this serious condition.

Defining factors that promote epileptogenesis in mouse models of MTLE-HS

We predict that AQP4-deficient mice are less prone to development of seizures than wild type mice and display reduced astroglial Ca^{2+} activity. We will use wild type mice and mice deficient in AQP4 or alpha-syntrophin (lacking perivascular AQP4) in order to determine whether AQP4 modulates the development of epileptogenic insults. Specifically, mice will be monitored for the development of spontaneous seizures and we will assess astroglial Ca^{2+} activity.

Assessment of the time course of alterations in Kir4.1 expression after status epilepticus (SE) in mouse model of MTLE-HS

In the chronical phase after SE, 'classical' astrocytes with passive current patterns are no longer present in the sclerotic CA1 region, closely resembling the situation in human HS. *This suggests the hypothesis that impairment of Kir4.1 function starts shortly after SE, during the latent period, and causes subsequent generation of spontaneous recurrent seizures*. Quantitative assessment of Kir4.1 expression of astrocytes post SE will be conducted. Preliminary data show that astrocytes increasingly lose gap junction coupling during this period. Whether this alteration is accompanied by reduced expression of Kir4.1 is therefore of interest.

Subgroup-specific pharmacoresistance in TLE

Patients with TLE frequently display pharmcoresistance and often require temporal lobectomy in order to achieve seizure control. One of the most important future tasks in overcoming medical intractability is identification of distinguishable entities among the TLE and tailoring pharmacological treatment to the respective TLE groups.

The extent to which different phenotypes of TLE are associated with pharmacoresistance to particular antiepileptic drugs (AEDs) has only been rudimentarily investigated.

Our TLE patient database contains detailed information about use of AED, including start and stop dates for 16 different AEDs, a rating scale for response to treatment, and other information. These data could serve as a useful basis for a comparative approach.

7. References

1. Abou-Khalil B, Krei L, Lazenby B, Harris PA, Haines JL, Hedera P. Familial genetic predisposition, epilepsy localization and antecedent febrile seizures. Epilepsy Res. 2007 Jan; 73 (1) :104-10.
2. Abrous DN, Koehl M, Le Moal M. Adult neurogenesis: from precursors to network and physiology. Physiol Rev. 2005 Apr; 85 (2) :523-69.
3. Amiry-Moghaddam M, Williamson A, Palomba M, Eid T, de Lanerolle NC, Nagelhus EA, Adams ME, Froehner SC, Agre P, Ottersen OP. Delayed K+ clearance associated with aquaporin-4 mislocalization: phenotypic defects in brains of alpha-syntrophin-null mice. Proc Natl Acad Sci U S A. 2003 Nov 11; 100 (23) :13615-20.
4. Amiry-Moghaddam M, Frydenlund DS, Ottersen OP. Anchoring of aquaporin-4 in brain: Molecular mechansisms and implications for the physiology and patophysiology of water transport. Neuroscience. 2004;129(4):999-1010.
5. Andersen, Per; Morris, Richard; Amaral, David Tim Bliss and O'Keefe, John; ed (2007). The Hippocampus Book. Oxford University Press. ISBN 978-0-19-510027-3
6. Angulo MC, Kozlov AS, Charpak S, Audinat E. Glutamate released from glial cells synchronizes neuronal activity in the hippocampus. J Neurosci. 2004 Aug 4; 24 (31) :6920-7.
7. Antman EM, Lau J, Kupelnick B, Mosteller F, Chalmers TC. A comparison of results of meta-analyses of randomized control trials and recommendations of clinical experts Treatments for myocardial infarction. JAMA. 1992 Jul 8; 268 (2) :240-8.
8. Araújo IM, Carreira BP, Carvalho CM, Carvalho AP. Calpains and delayed calcium deregulation in excitotoxicity. Neurochem Res. 2010 Dec; 35 (12) :1966-9.
9. Badaut J, Regli L. Distribution and possible roles of aquaporin 9 in the brain. Neuroscience. 2004; 129 (4) :971-81
10. Bailey P, Gibbs FA. The surgical theraphy of psychomotor epilepsy. JA M A. 1951;145:365–370
11. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005 Jan 15; 21 (2) :263-5
12. Baulac S, Picard F, Herman A, Feingold J, Genin E, Hirsch E, Prud'homme JF, Baulac M, Brice A, LeGuern E. Evidence for digenic inheritance in a family with both febrile convulsions and temporal lobe epilepsy implicating chromosomes 18qter and 1q25-q31. Ann Neurol. 2001 Jun; 49 (6) :786-92.
13. Baulac S, Huberfeld G, Gourfinkel-An I, Mitropoulou G, Beranger A, Prud'homme JF, Baulac M, Brice A, Bruzzone R, LeGuern E. First genetic evidence of GABA(A) receptor dysfunction in epilepsy: a mutation in the gamma2-subunit gene. Nat Genet. 2001 May; 28 (1) :46-8.
14. Baxendale SA, Van Paesschen W, Thompson PJ, Duncan JS, Harkness WF, Shorvon SD. Hippocampal cell loss and gliosis: relationship to preoperative and postoperative memory function. Neuropsychiatry Neuropsychol Behav Neurol. 1998 Jan; 11 (1) :12-21.
15. Bejerano G, Siepel AC, Kent WJ, Haussler D. Computational screening of conserved genomic DNA in search of functional noncoding elements. Nat Methods. 2005 Jul; 2 (7) :535-45.

16. Benbadis SR, Kotagal P, Klem GH. Unilateral blinking: a lateralizing sign in partial seizures. Neurology. 1996 Jan; 46 (1) :45-8.
17. Berg AT, Langfitt J, Shinnar S, Vickrey BG, Sperling MR, Walczak T, Bazil C, Pacia SV, Spencer SS. How long does it take for partial epilepsy to become intractable?. Neurology. 2003 Jan 28; 60 (2) :186-90.
18. Berg AT, Berkovic SF, Brodie MJ, Buchhalter J, Cross JH, van Emde Boas W, Engel J, French J, Glauser TA, Mathern GW, Moshé SL, Nordli D, Plouin P, Scheffer IE. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005-2009. Epilepsia. 2010 Apr; 51 (4) :676-85.
19. Bergles DE, Diamond JS, Jahr CE. Clearance of glutamate inside the synapse and beyond. Curr Opin Neurobiol. 1999 Jun; 9 (3) :293-8.
20. Berkovic SF, Howell RA, Hay DA, Hopper JL. Epilepsies in twins: genetics of the major epilepsy syndromes. Ann Neurol. 1998 Apr; 43 (4) :435-45.
21. Berkovic SF, McIntosh A, Howell RA, Mitchell A, Sheffield LJ, Hopper JL. Familial temporal lobe epilepsy: a common disorder identified in twins. Ann Neurol. 1996 Aug; 40 (2) :227-35.
22. Berkovic SF, McIntosh AM, Kalnins RM, Jackson GD, Fabinyi GC, Brazenor GA, Bladin PF, Hopper JL. Preoperative MRI predicts outcome of temporal lobectomy: an actuarial analysis. Neurology. 1995 Jul; 45 (7) :1358-63.
23. Binder DK, Steinhäuser C. Functional changes in astroglial cells in epilepsy. Glia. 2006 Oct; 54 (5) :358-68.
24. Bloch O, Papadopoulos MC, Manley GT, Verkman AS. Aquaporin-4 gene deletion in mice increases focal edema associated with staphylococcal brain abscess. J Neurochem. 2005 Oct; 95 (1) :254-62.
25. Bockenhauer D, Feather S, Stanescu HC, Bandulik S, Zdebik AA, Reichold M, Tobin J, Lieberer E, Sterner C, Landoure G, Arora R, Sirimanna T, Thompson D, Cross JH, van't Hoff W, Al Masri O, Tullus K, Yeung S, Anikster Y, Klootwijk E, Hubank M, Dillon MJ, Heitzmann D, Arcos-Burgos M, Knepper MA, Dobbie A, Gahl WA, Warth R, Sheridan E, Kleta R. Epilepsy, ataxia, sensorineural deafness, tubulopathy, and KCNJ10 mutations. N Engl J Med. 2009 May 7; 360 (19) :1960-70.
26. Bordey A, Sontheimer H. Properties of human glial cells associated with epileptic seizure foci. Epilepsy Res. 1998 Sep; 32 (1-2) :286-303.
27. Bouchet C, Cazauvieilh JB. De L'epilepsie consideree dans ses rapports avec Palienation mentale. Arch Gen Med. 1825;9:510-542
28. Bratz E. Ammonshornbefunde bei Epileptikern. Arch Psychiatr Nervenkr., 1899;32:820-835.
29. Briellmann RS, Torn-Broers Y, Jackson GD, Berkovic SF. Seizures in family members of patients with hippocampal sclerosis. Neurology. 2001 Nov 27; 57 (10) :1800-4. PubMed PMID:11723267.
30. Briellmann RS, Torn-Broers Y, Busuttil BE, Major BJ, Kalnins RM, Olsen M, Jackson GD, Frauman AG, Berkovic SF. APOE epsilon4 genotype is associated with an earlier onset of chronic temporal lobe epilepsy. Neurology. 2000 Aug 8; 55 (3) :435-7.
31. Brodtkorb E, Gu W, Nakken KO, Fischer C, Steinlein OK. Familial temporal lobe epilepsy with aphasic seizures and linkage to chromosome 10q22-q24. Epilepsia. 2002 Mar; 43 (3) :228-35.
32. Bruel-Jungerman E, Davis S, Laroche S. Brain plasticity mechanisms and memory: a party of four. Neuroscientist. 2007 Oct; 13 (5) :492-505.

33. Buono RJ, Ferraro TN, O'Connor MJ, Sperling MR, Ryan SG, Scattergood T, Mulholland N, Gilmore J, Lohoff FW, Berrettini WH. Lack of association between an interleukin 1 beta (IL-1beta) gene variation and refractory temporal lobe epilepsy. Epilepsia. 2001 Jun; 42 (6) :782-4.
34. Buono RJ, Lohoff FW, Sander T, Sperling MR, O'Connor MJ, Dlugos DJ, Ryan SG, Golden GT, Zhao H, Scattergood TM, Berrettini WH, Ferraro TN. Association between variation in the human KCNJ10 potassium ion channel gene and seizure susceptibility. Epilepsy Res. 2004 Feb; 58 (2-3) :175-83.
35. Butt AM, Kalsi A. Inwardly rectifying potassium channels (Kir) in central nervous system glia: a special role for Kir41 in glial functions. J Cell Mol Med. 2006 Jan-Mar; 10 (1) :33-44.
36. Camfield P, Camfield C, Gordon K, Dooley J. What types of epilepsy are preceded by febrile seizures? A population-based study of children. Dev Med Child Neurol. 1994 Oct; 36 (10) :887-92.
37. Caruncho, Manuel Vazquez and Fernandez, Franciso Branas. The hallucinations of F. Chopin, Med Humanities doi:10.1136/jmh.2010.005405
38. Cavalleri GL, Lynch JM, Depondt C, Burley MW, Wood NW, Sisodiya SM, Goldstein DB. Failure to replicate previously reported genetic associations with sporadic temporal lobe epilepsy: where to from here?. Brain. 2005 Aug; 128 (Pt 8) :1832-40.
39. Cendes F, Andermann F, Dubeau F, Gloor P, Evans A, Jones-Gotman M, Olivier A, Andermann E, Robitaille Y, Lopes-Cendes I. Early childhood prolonged febrile convulsions, atrophy and sclerosis of mesial structures, and temporal lobe epilepsy: an MRI volumetric study. Neurology. 1993 Jun; 43 (6) :1083-7.
40. Cendes F, Andermann F, Gloor P, Lopes-Cendes I, Andermann E, Melanson D, Jones-Gotman M, Robitaille Y, Evans A, Peters T. Atrophy of mesial structures in patients with temporal lobe epilepsy: cause or consequence of repeated seizures?. Ann Neurol. 1993 Dec; 34 (6) :795-801.
41. Charles AC, Merrill JE, Dirksen ER, Sanderson MJ. Intercellular signaling in glial cells: calcium waves and oscillations in response to mechanical stimulation and glutamate. Neuron. 1991 Jun; 6 (6) :983-92.
42. Chen Y, Zhu W, Bollen AW, Lawton MT, Barbaro NM, Dowd CF, Hashimoto T, Yang GY, Young WL. Evidence of inflammatory cell involvement in brain arteriovenous malformations. Neurosurgery. 2008 Jun; 62 (6) :1340-9; discussion 1349-50.
43. Chever O, Djukic B, McCarthy KD, Amzica F. Implication of Kir41 channel in excess potassium clearance: an in vivo study on anesthetized glial-conditional Kir41 knock-out mice. J Neurosci. 2010 Nov 24; 30 (47) :15769-77.
44. Claes L, Audenaert D, Deprez L, Van Paesschen W, Depondt C, Goossens D, Del-Favero J, Van Broeckhoven C, De Jonghe P. Novel locus on chromosome 12q22-q233 responsible for familial temporal lobe epilepsy associated with febrile seizures. J Med Genet. 2004 Sep; 41 (9) :710-4.
45. Colosimo E, Gambardella A, Mantegazza M, Labate A, Rusconi R, Schiavon E, Annesi F, Cassulini RR, Carrideo S, Chifari R, Canevini MP, Canger R, Franceschetti S, Annesi G, Wanke E, Quattrone A. Electroclinical features of a family with simple febrile seizures and temporal lobe epilepsy associated with SCN1A loss-of-function mutation. Epilepsia. 2007 Sep; 48 (9) :1691-6.
46. Connors NC, Adams ME, Froehner SC, Kofuji P. The potassium channel Kir41 associates with the dystrophin-glycoprotein complex via alpha-syntrophin in glia. J Biol Chem. 2004 Jul 2; 279 (27) :28387-92.

47. Contopoulos-Ioannidis DG, Ntzani E, Ioannidis JP. Translation of highly promising basic science research into clinical applications. Am J Med. 2003 Apr 15; 114 (6) :477-84.
48. Cordell HJ, Clayton DG. Genetic association studies. Lancet. 2005 Sep 24-30; 366 (9491) :1121-31. PubMed PMID:16182901.
49. Dalloz C, Sarig R, Fort P, Yaffe D, Bordais A, Pannicke T, Grosche J, Mornet D, Reichenbach A, Sahel J, Nudel U, Rendon A. Targeted inactivation of dystrophin gene product Dp71: phenotypic impact in mouse retina. Hum Mol Genet. 2003 Jul 1; 12 (13) :1543-54.
50. Danbolt NC. Glutamate uptake. Prog Neurobiol. 2001 Sep; 65 (1) :1-105.
51. Davies KG, Hermann BP, Dohan FC Jr, Foley KT, Bush AJ, Wyler AR. Relationship of hippocampal sclerosis to duration and age of onset of epilepsy, and childhood febrile seizures in temporal lobectomy patients. Epilepsy Res. 1996 Jun; 24 (2) :119-26.
52. Dibbens LM, Feng HJ, Richards MC, Harkin LA, Hodgson BL, Scott D, Jenkins M, Petrou S, Sutherland GR, Scheffer IE, Berkovic SF, Macdonald RL, Mulley JC. GABRD encoding a protein for extra- or peri-synaptic GABAA receptors is a susceptibility locus for generalized epilepsies. Hum Mol Genet. 2004 Jul 1; 13 (13) :1315-9.
53. Dietzel I, Heinemann U, Hofmeier G, Lux HD. Stimulus-induced changes in extracellular Na+ and Cl- concentration in relation to changes in the size of the extracellular space. Exp Brain Res. 1982; 46 (1) :73-84.
54. Djukic B, Casper KB, Philpot BD, Chin LS, McCarthy KD. Conditional knock-out of Kir41 leads to glial membrane depolarization, inhibition of potassium and glutamate uptake, and enhanced short-term synaptic potentiation. J Neurosci. 2007 Oct 17; 27 (42) :11354-65.
55. Doupnik CA, Davidson N, Lester HA. The inward rectifier potassium channel family. Curr Opin Neurobiol. 1995 Jun; 5 (3) :268-77.
56. Dubé CM, Ravizza T, Hamamura M, Zha Q, Keebaugh A, Fok K, Andres AL, Nalcioglu O, Obenaus A, Vezzani A, Baram TZ. Epileptogenesis provoked by prolonged experimental febrile seizures: mechanisms and biomarkers. J Neurosci. 2010 Jun 2; 30 (22) :7484-94.
57. Duncan JS, Sagar HJ. Seizure characteristics, pathology, and outcome after temporal lobectomy. Neurology. 1987 Mar; 37 (3) :405-9.
58. Eid T, Lee TS, Thomas MJ, Amiry-Moghaddam M, Bjørnsen LP, Spencer DD, Agre P, Ottersen OP, de Lanerolle NC. Loss of perivascular aquaporin 4 may underlie deficient water and K+ homeostasis in the human epileptogenic hippocampus. Proc Natl Acad Sci U S A. 2005 Jan 25; 102 (4) :1193-8. PubMed PMID:15657133;
59. Eid T, Williamson A, Lee TS, Petroff OA, de Lanerolle NC. Glutamate and astrocytes--key players in human mesial temporal lobe epilepsy?. Epilepsia. 2008; 49 Suppl 2:42-52.
60. Eisch AJ, Cameron HA, Encinas JM, Meltzer LA, Ming GL, Overstreet-Wadiche LS. Adult neurogenesis, mental health, and mental illness: hope or hype?. J Neurosci. 2008 Nov 12; 28 (46) :11785-91.
61. Engel J Jr, Wiebe S, French J, Sperling M, Williamson P, Spencer D, Gumnit R, Zahn C, Westbrook E, Enos B. Practice parameter: temporal lobe and localized neocortical resections for epilepsy. Epilepsia. 2003 Jun; 44 (6) :741-51.

62. Escayg A, MacDonald BT, Meisler MH, Baulac S, Huberfeld G, An-Gourfinkel I, Brice A, LeGuern E, Moulard B, Chaigne D, Buresi C, Malafosse A. Mutations of SCN1A, encoding a neuronal sodium channel, in two families with GEFS+2. Nat Genet. 2000 Apr; 24 (4) :343-5.
63. Esteller M,The Lancet, Volume 372, Pages S90 - S96,1 December.2008;doi:10.1016/S0140-6736(08)61887-5
64. Falconer MA. Discussion on the surgery of temporal lobe epilepsy: surgical and pathological aspects. Proc R Soc Med. 1953;46:971-974
65. Farrow TF, Dickson JM, Grünewald RA. A six-year follow-up MRI study of complicated early childhood convulsion. Pediatr Neurol. 2006 Oct; 35 (4) :257-60.
66. French JA, Williamson PD, Thadani VM, Darcey TM, Mattson RH, Spencer SS, Spencer DD. Characteristics of medial temporal lobe epilepsy: I Results of history and physical examination. Ann Neurol. 1993 Dec; 34 (6) :774-80.
67. Fukata Y, Lovero KL, Iwanaga T, Watanabe A, Yokoi N, Tabuchi K, Shigemoto R, Nicoll RA, Fukata M. Disruption of LGI1-linked synaptic complex causes abnormal synaptic transmission and epilepsy. Proc Natl Acad Sci U S A. 2010 Feb 23; 107 (8) :3799-804.
68. Fullerton SM, Yu JH, Crouch J, Fryer-Edwards K, Burke W. Population description and its role in the interpretation of genetic association. Hum Genet. 2010 Mar; 127 (5) :563-72.
69. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D. The structure of haplotype blocks in the human genome. Science. 2002 Jun 21; 296 (5576) :2225-9.
70. Gaitatzis A, Trimble MR, Sander JW. The psychiatric comorbidity of epilepsy. Acta Neurol Scand. 2004 Oct; 110 (4) :207-20.
71. Gambardella A, Labate A, Giallonardo A, Aguglia U. Familial mesial temporal lobe epilepsies: clinical and genetic features. Epilepsia. 2009 May; 50 Suppl 5:55-7.
72. Gambardella A, Manna I, Labate A, Chifari R, La Russa A, Serra P, Cittadella R, Bonavita S, Andreoli V, LePiane E, Sasanelli F, Di Costanzo A, Zappia M, Tedeschi G, Aguglia U, Quattrone A. GABA(B) receptor 1 polymorphism (G1465A) is associated with temporal lobe epilepsy. Neurology. 2003 Feb 25; 60 (4) :560-3.
73. Gibbs FA, Gibbs EL, Lennox WG. Epilepsy: a paroxysmal cerebral dysrhythmia. Brain 1937;60(4):377-88.
74. Goldsmith IL*,* Buchhalter JR. Incidence of hippocampal sclerosis in Rochester Minnesota *1990–1996.* Epilepsia 2002*;*43*(*Suppl. 7*):*163 *(Abst. 2.103).*
75. Goodwin F, Muntoni F, Dubowitz V. Epilepsy in Duchenne and Becker muscular dystrophies. Eur J Paediatr Neurol. 1997; 1 (4) :115-9.
76. Greenberg DA, Subaran R. Blinders, phenotype, and fashionable genetic analysis: a critical examination of the current state of epilepsy genetic studies. Epilepsia. 2011 Jan; 52 (1) :1-9.
77. Hamati-Haddad A, Abou-Khalil B. Epilepsy diagnosis and localization in patients with antecedent childhood febrile convulsions. Neurology. 1998 Apr; 50 (4) :917-22.
78. Hastie T, Tibshirani R, Friedman J. The Elements of Statistical Learning: Data Mining, Inference and Prediction, Springer, New York (2001).

79. Hauser WA. The prevalence and incidence of convulsive disorders in children. Epilepsia. 1994; 35 Suppl 2:S1-6.
80. Hauser WA, Annegers JF, Rocca WA. Descriptive epidemiology of epilepsy: contributions of population-based studies from Rochester, Minnesota. Mayo Clin Proc. 1996 Jun; 71 (6) :576-86.
81. Hauser WA, Annegers JF, Anderson VE, Kurland LT. The risk of seizure disorders among relatives of children with febrile convulsions. Neurology. 1985 Sep; 35 (9) :1268-73.
82. Hayashi T, Kaneko Y, Yu S, Bae E, Stahl CE, Kawase T, van Loveren H, Sanberg PR, Borlongan CV. Quantitative analyses of matrix metalloproteinase activity after traumatic brain injury in adult rats. Brain Res. 2009 Jul 14; 1280:172-7.
83. Haydon PG. GLIA: listening and talking to the synapse. Nat Rev Neurosci. 2001 Mar; 2 (3) :185-93.
84. Hedera P, Blair MA, Andermann E, Andermann F, D'Agostino D, Taylor KA, Chahine L, Pandolfo M, Bradford Y, Haines JL, Abou-Khalil B. Familial mesial temporal lobe epilepsy maps to chromosome 4q132-q213. Neurology. 2007 Jun 12; 68 (24) :2107-12.
85. Heils A, Haug K, Kunz WS, Fernandez G, Horvath S, Rebstock J, Propping P, Elger CE. Interleukin-1beta gene polymorphism and susceptibility to temporal lobe epilepsy with hippocampal sclerosis. Ann Neurol. 2000 Dec; 48 (6) :948-50.
86. Helbig I, Scheffer IE, Mulley JC, Berkovic SF. Navigating the channels and beyond: unravelling the genetics of the epilepsies. Lancet Neurol. 2008 Mar; 7 (3) :231-45.
87. Helmstaedter C, Sonntag-Dillender M, Hoppe C, Elger CE. Depressed mood and memory impairment in temporal lobe epilepsy as a function of focus lateralization and localization. Epilepsy Behav. 2004 Oct; 5 (5) :696-701.
88. Hermann BP, Seidenberg M, Schoenfeld J, Davies K. Neuropsychological characteristics of the syndrome of mesial temporal lobe epilepsy. Arch Neurol. 1997 Apr; 54 (4) :369-76.
89. Heuser K, Nagelhus EA, Taubøll E, Indahl U, Berg PR, Lien S, Nakken S, Gjerstad L, Ottersen OP. Variants of the genes encoding AQP4 and Kir41 are associated with subgroups of patients with temporal lobe epilepsy. Epilepsy Res. 2010 Jan; 88 (1) :55-64.
90. Heuser K, Cvancarova M, Gjerstad L, Taubøll E. Is Temporal Lobe Epilepsy with childhood febrile seizures a distinctive entity? A comparative study. Seizure. 2011 Mar; 20 (2) :163-6.
91. Heuser K, Hoddevik EH, Taubøll E, Gjerstad L, Indahl U, Kaczmarek L, Berg PR, Lien S, Nagelhus EA, Ottersen OP. Temporal lobe epilepsy and matrix metalloproteinase 9: a tempting relation but negative genetic association. Seizure. 2010 Jul; 19 (6) :335-8
92. Higashi K, Fujita A, Inanobe A, Tanemoto M, Doi K, Kubo T, Kurachi Y. An inwardly rectifying K(+) channel, Kir41, expressed in astrocytes surrounds synapses and blood vessels in brain. Am J Physiol Cell Physiol. 2001 Sep; 281 (3) :C922-31.
93. Hindorff LA, Sethupathy P, Junkins HA, Ramos EM, Mehta JP, Collins FS, Manolio TA. Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. Proc Natl Acad Sci U S A. 2009 Jun 9; 106 (23) :9362-7.

94. Hinterkeuser S, Schröder W, Hager G, Seifert G, Blümcke I, Elger CE, Schramm J, Steinhäuser C. Astrocytes in the hippocampus of patients with temporal lobe epilepsy display changes in potassium conductances. Eur J Neurosci. 2000 Jun; 12 (6) :2087-96.
95. Hippocrates. On the sacred disease. In: Page TE, Capps E, Rouse WHD, et al., eds. Hippocrates. Vol. 2. Cambridge, MA: Havard University Press; 1967: 127-184.
96. Hirschhorn JN, Lohmueller K, Byrne E, Hirschhorn K. A comprehensive review of genetic association studies. Genet Med. 2002 Mar-Apr; 4 (2) :45-61.
97. Hogan RE, Kaiboriboon K. The "dreamy state": John Hughlings-Jackson's ideas of epilepsy and consciousness. Am J Psychiatry. 2003 Oct; 160 (10) :1740-7.
98. Hsu SM, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem. 1981 Apr; 29 (4) :577-80.
99. Hugg JW, Butterworth EJ, Kuzniecky RI. Diffusion mapping applied to mesial temporal lobe epilepsy: preliminary observations. Neurology. 1999 Jul 13; 53 (1) :173-6.
100. Ichiyama T, Takahashi Y, Matsushige T, Kajimoto M, Fukunaga S, Furukawa S. Serum matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 levels in non-herpetic acute limbic encephalitis. J Neurol. 2009 Nov; 256 (11) :1846-50.
101. Illarionova NB, Gunnarson E, Li Y, Brismar H, Bondar A, Zelenin S, Aperia A. Functional and molecular interactions between aquaporins and Na,K-ATPase. Neuroscience. 2010 Jul 28; 168 (4) :915-25.
102. Indahl U. A twist to partial least squares regression, *J. Chemometr.* **19** (2005), pp. 32–44
103. Ioannidis JP. Materializing research promises: opportunities, priorities and conflicts in translational medicine. J Transl Med. 2004 Jan 31; 2 (1) :5.
104. Ishii M, Horio Y, Tada Y, Hibino H, Inanobe A, Ito M, Yamada M, Gotow T, Uchiyama Y, Kurachi Y. Expression and clustered distribution of an inwardly rectifying potassium channel, KAB-2/Kir41, on mammalian retinal Müller cell membrane: their regulation by insulin and laminin signals. J Neurosci. 1997 Oct 15; 17 (20) :7725-35.
105. Jabs R, Seifert G, Steinhäuser C. Astrocytic function and its alteration in the epileptic brain. Epilepsia. 2008; 49 Suppl 2:3-12.
106. Jackson JH. On a particular variety of epilepsy ("intellectual aura"), one case with symptoms of organic brain disease. Brain 1880;11:179-207.
107. Jackson JH, Colman WS. Case of epilepsy with tasting movements and "dreaming state"—very small patch of softening in the left uncinate gyrus. Brain 1898;21:580-90
108. Janszky J, Janszky I, Ebner A. Age at onset in mesial temporal lobe epilepsy with a history of febrile seizures. Neurology. 2004 Oct 12; 63 (7) :1296-8.
109. Jasper H, Kershman J. Electroencephalographic classification of the epilepsies. Archives of Neurology and Psychiatry 1941;45:903-43.
110. Jensen FE, Baram TZ. Developmental seizures induced by common early-life insults: short- and long-term effects on seizure susceptibility. Ment Retard Dev Disabil Res Rev. 2000; 6 (4) :253-7.
111. Johnson MR, Sander JW. The clinical impact of epilepsy genetics. J Neurol Neurosurg Psychiatry. 2001 Apr; 70 (4) :428-30. PubMed PMID:11254761.

112. Johnson WG, Kugler SL, Stenroos ES, Meulener MC, Rangwalla I, Johnson TW, Mandelbaum DE. Pedigree analysis in families with febrile seizures. Am J Med Genet. 1996 Feb 2; 61 (4) :345-52.
113. Johnston D. Valproic acid: update on its mechanisms of action. Epilepsia. 1984; 25 Suppl 1:S1-4.
114. Jones T, Jacobsen SJ. Childhood febrile seizures: overview and implications. Int J Med Sci. 2007 Apr 7; 4 (2) :110-4.
115. Jung JS, Bhat RV, Preston GM, Guggino WB, Baraban JM, Agre P. Molecular characterization of an aquaporin cDNA from brain: candidate osmoreceptor and regulator of water balance. Proc Natl Acad Sci U S A. 1994 Dec 20; 91 (26) :13052-6.
116. Kalachikov S, Evgrafov O, Ross B, Winawer M, Barker-Cummings C, Martinelli Boneschi F, Choi C, Morozov P, Das K, Teplitskaya E, Yu A, Cayanis E, Penchaszadeh G, Kottmann AH, Pedley TA, Hauser WA, Ottman R, Gilliam TC. Mutations in LGI1 cause autosomal-dominant partial epilepsy with auditory features. Nat Genet. 2002 Mar; 30 (3) :335-41.
117. Kalsi AS, Greenwood K, Wilkin G, Butt AM. Kir41 expression by astrocytes and oligodendrocytes in CNS white matter: a developmental study in the rat optic nerve. J Anat. 2004 Jun; 204 (6) :475-85.
118. Kanemoto K, Kawasaki J, Miyamoto T, Obayashi H, Nishimura M. Interleukin (IL)1beta, IL-1alpha, and IL-1 receptor antagonist gene polymorphisms in patients with temporal lobe epilepsy. Ann Neurol. 2000 May; 47 (5) :571-4.
119. Kaplan JB, Bennett T. Use of race and ethnicity in biomedical publication. JAMA. 2003 May 28; 289 (20) :2709-16.
120. Karenberg A. Chopin's misery and musicians' medical biography. Sudhoffs Arch. 2007; 91 (1) :82-98.
121. Karolchik D, Kuhn RM, Baertsch R, Barber GP, Clawson H, Diekhans M, Giardine B, Harte RA, Hinrichs AS, Hsu F, Kober KM, Miller W, Pedersen JS, Pohl A, Raney BJ, Rhead B, Rosenbloom KR, Smith KE, Stanke M, Thakkapallayil A, Trumbower H, Wang T, Zweig AS, Haussler D, Kent WJ. The UCSC Genome Browser Database: 2008 update. Nucleic Acids Res. 2008 Jan; 36 (Database issue) :D773-9.
122. Kasperaviciūte D, Catarino CB, Heinzen EL, Depondt C, Cavalleri GL, Caboclo LO, Tate SK, Jamnadas-Khoda J, Chinthapalli K, Clayton LM, Shianna KV, Radtke RA, Mikati MA, Gallentine WB, Husain AM, Alhusaini S, Leppert D, Middleton LT, Gibson RA, Johnson MR, Matthews PM, Hosford D, Heuser K, Amos L, Ortega M, Zumsteg D, Wieser HG, Steinhoff BJ, Krämer G, Hansen J, Dorn T, Kantanen AM, Gjerstad L, Peuralinna T, Hernandez DG, Eriksson KJ, Kälviäinen RK, Doherty CP, Wood NW, Pandolfo M, Duncan JS, Sander JW, Delanty N, Goldstein DB, Sisodiya SM. Common genetic variation and susceptibility to partial epilepsies: a genome-wide association study. Brain. 2010 Jul; 133 (Pt 7) :2136-47.
123. Kempermann G, Jessberger S, Steiner B, Kronenberg G. Milestones of neuronal development in the adult hippocampus. Trends Neurosci. 2004 Aug; 27 (8) :447-52.
124. Kobayashi E, D'Agostino MD, Lopes-Cendes I, Berkovic SF, Li ML, Andermann E, Andermann F, Cendes F. Hippocampal atrophy and T2-weighted signal changes in familial mesial temporal lobe epilepsy. Neurology. 2003 Feb 11; 60 (3) :405-9.

125. Kobayashi E, Li LM, Lopes-Cendes I, Cendes F. Magnetic resonance imaging evidence of hippocampal sclerosis in asymptomatic, first-degree relatives of patients with familial mesial temporal lobe epilepsy. Arch Neurol. 2002 Dec; 59 (12) :1891-4.
126. Kofuji P, Newman EA. Potassium buffering in the central nervous system. Neuroscience. 2004; 129 (4) :1045-56.
127. Kofuji P, Ceelen P, Zahs KR, Surbeck LW, Lester HA, Newman EA. Genetic inactivation of an inwardly rectifying potassium channel (Kir41 subunit) in mice: phenotypic impact in retina. J Neurosci. 2000 Aug 1; 20 (15) :5733-40.
128. Kotagal P, Lüders H, Morris HH, Dinner DS, Wyllie E, Godoy J, Rothner AD. Dystonic posturing in complex partial seizures of temporal lobe onset: a new lateralizing sign. Neurology. 1989 Feb; 39 (2 Pt 1) :196-201.
129. Kramer RE, Lüders H, Goldstick LP, Dinner DS, Morris HH, Lesser RP, Wyllie E. Ictus emeticus: an electroclinical analysis. Neurology. 1988 Jul; 38 (7) :1048-52.
130. Krapivinsky G, Gordon EA, Wickman K, Velimirović B, Krapivinsky L, Clapham DE. The G-protein-gated atrial K+ channel IKACh is a heteromultimer of two inwardly rectifying K(+)-channel proteins. Nature. 1995 Mar 9; 374 (6518) :135-41.
131. Kuffler SW, Nicholls JG, Orkand RK. Physiological properties of glial cells in the central nervous system of amphibia. J Neurophysiol. 1966 Jul; 29 (4) :768-87.
132. Kwan P, Brodie MJ. Early identification of refractory epilepsy. N Engl J Med. 2000 Feb 3; 342 (5) :314-9.
133. Lai M, Huijbers MG, Lancaster E, Graus F, Bataller L, Balice-Gordon R, Cowell JK, Dalmau J. Investigation of LGI1 as the antigen in limbic encephalitis previously attributed to potassium channels: a case series. Lancet Neurol. 2010 Aug; 9 (8) :776-85.
134. Lander ES, Schork NJ. Genetic dissection of complex traits. Science. 1994 Sep 30; 265 (5181) :2037-48.
135. Lederfein D, Levy Z, Augier N, Mornet D, Morris G, Fuchs O, Yaffe D, Nudel U. A 71-kilodalton protein is a major product of the Duchenne muscular dystrophy gene in brain and other nonmuscle tissues. Proc Natl Acad Sci U S A. 1992 Jun 15; 89 (12) :5346-50. P
136. Lee DH, Gao FQ, Rogers JM, Gulka I, Mackenzie IR, Parrent AG, Kubu CS, Munoz DG, McLachlan RS, Blume WT, Girvin JP. MR in temporal lobe epilepsy: analysis with pathologic confirmation. AJNR Am J Neuroradiol. 1998 Jan; 19 (1) :19-27.
137. Lee SK, Kim KK, Hong KS, Kim JY, Chung CK. The lateralizing and surgical prognostic value of a single 2-hour EEG in mesial TLE. Seizure. 2000 Jul; 9 (5) :336-9.
138. Lennox WG, Lennox MA. The genetics of epilepsy. Epilepsy and related disorders. Vol 1. Boston: Little, Brown, 1960:532.574
139. Lenzen KP, Heils A, Lorenz S, Hempelmann A, Höfels S, Lohoff FW, Schmitz B, Sander T. Supportive evidence for an allelic association of the human KCNJ10 potassium channel gene with idiopathic generalized epilepsy. Epilepsy Res. 2005 Feb; 63 (2-3) :113-8.
140. Leppert D, Leib SL, Grygar C, Miller KM, Schaad UB, Holländer GA. Matrix metalloproteinase (MMP)-8 and MMP-9 in cerebrospinal fluid during bacterial meningitis: association with blood-brain barrier damage and neurological sequelae. Clin Infect Dis. 2000 Jul; 31 (1) :80-4.

141. Liu JW, Wakayama Y, Inoue M, Shibuya S, Kojima H, Jimi T, Oniki H. Immunocytochemical studies of aquaporin 4 in the skeletal muscle of mdx mouse. J Neurol Sci. 1999 Mar 15; 164 (1) :24-8.
142. Löscher W. Effects of the antiepileptic drug valproate on metabolism and function of inhibitory and excitatory amino acids in the brain. Neurochem Res. 1993 Apr; 18 (4) :485-502.
143. Maher J, McLachlan RS. Febrile convulsions Is seizure duration the most important predictor of temporal lobe epilepsy?. Brain. 1995 Dec; 118 (Pt 6):1521-8.
144. Manley GT, Fujimura M, Ma T, Noshita N, Filiz F, Bollen AW, Chan P, Verkman AS. Aquaporin-4 deletion in mice reduces brain edema after acute water intoxication and ischemic stroke. Nat Med. 2000 Feb; 6 (2) :159-63.
145. Marincola FM. Translational Medicine: A two-way road. J Transl Med. 2003 Jul 24; 1 (1) :1. PubMed PMID:14527344;
146. Martens H and Martens M. Multivariate Analysis of Quality: An Introduction, Wiley, Chichester, UK (2001).
147. Mathern GW, Babb TL, Vickrey BG, Melendez M, Pretorius JK. The clinical-pathogenic mechanisms of hippocampal neuron loss and surgical outcomes in temporal lobe epilepsy. Brain. 1995 Feb; 118 (Pt 1):105-18.
148. McCauley JL, Kenealy SJ, Margulies EH, Schnetz-Boutaud N, Gregory SG, Hauser SL, Oksenberg JR, Pericak-Vance MA, Haines JL, Mortlock DP. SNPs in Multi-species Conserved Sequences (MCS) as useful markers in association studies: a practical approach. BMC Genomics. 2007 Aug 6; 8:266
149. McClelland S, Dubé CM, Yang J, Baram TZ. Epileptogenesis after prolonged febrile seizures: Mechanisms, biomarkers and therapeutic opportunities. Neurosci Lett. 2011 Jun 27; 497 (3) :155-62.
150. McIntosh AM, Wilson SJ, Berkovic SF. Seizure outcome after temporal lobectomy: current research practice and findings. Epilepsia. 2001 Oct; 42 (10) :1288-307.
151. Mendez MF, Cummings JL, Benson DF. Depression in epilepsy Significance and phenomenology. Arch Neurol. 1986 Aug; 43 (8) :766-70.
152. Michaluk P, Kaczmarek L. Matrix metalloproteinase-9 in glutamate-dependent adult brain function and dysfunction. Cell Death Differ. 2007 Jul; 14 (7) :1255-8.
153. Millichap JG, Millichap JJ. Role of viral infections in the etiology of febrile seizures. Pediatr Neurol. 2006 Sep; 35 (3) :165-72.
154. Nagelhus EA, Horio Y, Inanobe A, Fujita A, Haug FM, Nielsen S, Kurachi Y, Ottersen OP. Immunogold evidence suggests that coupling of K+ siphoning and water transport in rat retinal Müller cells is mediated by a coenrichment of Kir41 and AQP4 in specific membrane domains. Glia. 1999 Mar; 26 (1) :47-54.
155. Nagelhus EA, Mathiisen TM, Ottersen OP. Aquaporin-4 in the central nervous system: cellular and subcellular distribution and coexpression with KIR4.1. Neuroscience. 2004; 129 (4) :905-13.
156. Nagy V, Bozdagi O, Matynia A, Balcerzyk M, Okulski P, Dzwonek J, Costa RM, Silva AJ, Kaczmarek L, Huntley GW. Matrix metalloproteinase-9 is required for hippocampal late-phase long-term potentiation and memory. J Neurosci. 2006 Feb 15; 26 (7) :1923-34.

157. Nakayama J, Hamano K, Noguchi E, Horiuchi Y, Iwasaki N, Ohta M, Nakahara S, Naoi T, Matsui A, Arinami T. Failure to find causal mutations in the GABA(A)-receptor gamma2 subunit (GABRG2) gene in Japanese febrile seizure patients. Neurosci Lett. 2003 Jun 5; 343 (2) :117-20.
158. Nakayama J. Progress in searching for the febrile seizure susceptibility genes. Brain Dev. 2009 May; 31 (5) :359-65.
159. Nedergaard M, Verkhratsky A. Calcium dyshomeostasis and pathological calcium signalling in neurological diseases. Cell Calcium. 2010 Feb; 47 (2) :101-2. PubMed PMID:20079921.
160. Nichols CG, Lopatin AN. Inward rectifier potassium channels. Annu Rev Physiol. 1997; 59:171-91.
161. Nico B, Frigeri A, Nicchia GP, Corsi P, Ribatti D, Quondamatteo F, Herken R, Girolamo F, Marzullo A, Svelto M, Roncali L. Severe alterations of endothelial and glial cells in the blood-brain barrier of dystrophic mdx mice. Glia. 2003 May; 42 (3) :235-51.
162. Nielsen S, Smith BL, Christensen EI, Agre P. Distribution of the aquaporin CHIP in secretory and resorptive epithelia and capillary endothelia. Proc Natl Acad Sci U S A. 1993 Aug 1; 90 (15) :7275-9.
163. Nielsen S, Nagelhus EA, Amiry-Moghaddam M, Bourque C, Agre P, Ottersen OP. Specialized membrane domains for water transport in glial cells: high-resolution immunogold cytochemistry of aquaporin-4 in rat brain. J Neurosci. 1997 Jan 1; 17 (1) :171-80.
164. Niermann H, Amiry-Moghaddam M, Holthoff K, Witte OW, Ottersen OP. A novel role of vasopressin in the brain: modulation of activity-dependent water flux in the neocortex. J Neurosci. 2001 May 1; 21 (9) :3045-51.
165. Nobile C, Michelucci R, Andreazza S, Pasini E, Tosatto SC, Striano P. LGI1 mutations in autosomal dominant and sporadic lateral temporal epilepsy. Hum Mutat. 2009 Apr; 30 (4) :530-6.
166. Nocairi H, El Qannari M, Vigneau E, Bertrand D. Discrimination on latent components with respect to patterns—application to multicollinear data, *Comput. Stat. Data Anal.* **48** (2005), pp. 139–147
167. Ohmori I, Ouchida M, Ohtsuka Y, Oka E, Shimizu K. Significant correlation of the SCN1A mutations and severe myoclonic epilepsy in infancy. Biochem Biophys Res Commun. 2002 Jul 5; 295 (1) :17-23.
168. Olsen ML, Sontheimer H. Functional implications for Kir41 channels in glial biology: from K+ buffering to cell differentiation. J Neurochem. 2008 Nov; 107 (3) :589-601.
169. Orkand RK. Extracellular potassium accumulation in the nervous system. Fed Proc. 1980 Apr; 39 (5) :1515-8. PubMed PMID:7364046.
170. Ottman R, Risch N, Hauser WA, Pedley TA, Lee JH, Barker-Cummings C, Lustenberger A, Nagle KJ, Lee KS, Scheuer ML. Localization of a gene for partial epilepsy to chromosome 10q. Nat Genet. 1995 May; 10 (1) :56-60.
171. Padmawar P, Yao X, Bloch O, Manley GT, Verkman AS. K+ waves in brain cortex visualized using a long-wavelength K+-sensing fluorescent indicator. Nat Methods. 2005 Nov; 2 (11) :825-7.
172. Papadopoulos MC, Verkman AS. Aquaporin-4 gene disruption in mice reduces brain swelling and mortality in pneumococcal meningitis. J Biol Chem. 2005 Apr 8; 280 (14) :13906-12.
173. Parent JM. Adult neurogenesis in the intact and epileptic dentate gyrus. Prog Brain Res. 2007; 163:529-40.

174. Park KP, Rosell A, Foerch C, Xing C, Kim WJ, Lee S, Opdenakker G, Furie KL, Lo EH. Plasma and brain matrix metalloproteinase-9 after acute focal cerebral ischemia in rats. Stroke. 2009 Aug; 40 (8) :2836-42.
175. Parpura V, Basarsky TA, Liu F, Jeftinija K, Jeftinija S, Haydon PG. Glutamate-mediated astrocyte-neuron signalling. Nature. 1994 Jun 30; 369 (6483) :744-7
176. Peters MF, Adams ME, Froehner SC. Differential association of syntrophin pairs with the dystrophin complex. J Cell Biol. 1997 Jul 14; 138 (1) :81-93
177. Phillips PC. Epistasis--the essential role of gene interactions in the structure and evolution of genetic systems. Nat Rev Genet. 2008 Nov; 9 (11) :855-67.
178. Pisacane A, Sansone R, Impagliazzo N, Coppola A, Rolando P, D'Apuzzo A, Tregrossi C. Iron deficiency anaemia and febrile convulsions: case-control study in children under 2 years. BMJ. 1996 Aug 10; 313 (7053) :343.
179. Pitkänen A, Lukasiuk K. Molecular and cellular basis of epileptogenesis in symptomatic epilepsy. Epilepsy Behav. 2009 Jan; 14 Suppl 1:16-25.
180. Portela A, Esteller M. Epigenetic modifications and human disease. Nat Biotechnol. 2010 Oct; 28 (10) :1057-68.
181. Porter JT, McCarthy KD. Hippocampal astrocytes in situ respond to glutamate released from synaptic terminals. J Neurosci. 1996 Aug 15; 16 (16) :5073-81.
182. Poza JJ, Sáenz A, Martínez-Gil A, Cheron N, Cobo AM, Urtasun M, Martí-Massó JF, Grid D, Beckmann JS, Prud'homme JF, López de Munain A. Autosomal dominant lateral temporal epilepsy: clinical and genetic study of a large Basque pedigree linked to chromosome 10q. Ann Neurol. 1999 Feb; 45 (2) :182-8.
183. Preston GM, Agre P. Isolation of the cDNA for erythrocyte integral membrane protein of 28 kilodaltons: member of an ancient channel family. Proc Natl Acad Sci U S A. 1991 Dec 15; 88 (24) :11110-4.
184. Proposal for classification of epilepsies and epileptic syndromes Commission on Classification and Terminology of the International League Against Epilepsy. Epilepsia. 1985 May-Jun; 26 (3) :268-78.
185. Proposal for revised classification of epilepsies and epileptic syndromes Commission on Classification and Terminology of the International League Against Epilepsy. Epilepsia. 1989 Jul-Aug; 30 (4) :389-99.
186. Quiske A, Helmstaedter C, Lux S, Elger CE. Depression in patients with temporal lobe epilepsy is related to mesial temporal sclerosis. Epilepsy Res. 2000 Apr; 39 (2) :121-5.
187. Reid CA, Berkovic SF, Petrou S. Mechanisms of human inherited epilepsies. Prog Neurobiol. 2009 Jan 12; 87 (1) :41-57.
188. Rich SS, Annegers JF, Hauser WA, Anderson VE. Complex segregation analysis of febrile convulsions. Am J Hum Genet. 1987 Aug; 41 (2) :249-57.
189. Rogawski MA, Löscher W. The neurobiology of antiepileptic drugs for the treatment of nonepileptic conditions. Nat Med. 2004 Jul; 10 (7) :685-92.
190. Salzmann A, Perroud N, Crespel A, Lambercy C, Malafosse A. Candidate genes for temporal lobe epilepsy: a replication study. Neurol Sci. 2008 Dec; 29 (6) :397-403
191. Sander JW, Shorvon SD. Incidence and prevalence studies in epilepsy and their methodological problems: a review. J Neurol Neurosurg Psychiatry. 1987 Jul; 50 (7) :829-39.

192. Sawrie SM, Martin RC, Knowlton R, Faught E, Gilliam F, Kuzniecky R. Relationships among hippocampal volumetry, proton magnetic resonance spectroscopy, and verbal memory in temporal lobe epilepsy. Epilepsia. 2001 Nov; 42 (11) :1403-7.
193. Saygi S, Spencer SS, Scheyer R, Katz A, Mattson R, Spencer DD. Differentiation of temporal lobe ictal behavior associated with hippocampal sclerosis and tumors of temporal lobe. Epilepsia. 1994 Jul-Aug; 35 (4) :737-42.
194. Scantlebury MH, Heida JG. Febrile seizures and temporal lobe epileptogenesis. Epilepsy Res. 2010 Mar; 89 (1) :27-33.
195. Scheffer IE, Berkovic SF. Generalized epilepsy with febrile seizures plus A genetic disorder with heterogeneous clinical phenotypes. Brain. 1997 Mar; 120 (Pt 3):479-90.
196. Scheffer IE, Harkin LA, Grinton BE, Dibbens LM, Turner SJ, Zielinski MA, Xu R, Jackson G, Adams J, Connellan M, Petrou S, Wellard RM, Briellmann RS, Wallace RH, Mulley JC, Berkovic SF. Temporal lobe epilepsy and GEFS+ phenotypes associated with SCN1B mutations. Brain. 2007 Jan; 130 (Pt 1) :100-9.
197. Scholl UI, Choi M, Liu T, Ramaekers VT, Häusler MG, Grimmer J, Tobe SW, Farhi A, Nelson-Williams C, Lifton RP. Seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte imbalance (SeSAME syndrome) caused by mutations in KCNJ10. Proc Natl Acad Sci U S A. 2009 Apr 7; 106 (14) :5842-7.
198. Schröder W, Hinterkeuser S, Seifert G, Schramm J, Jabs R, Wilkin GP, Steinhäuser C. Functional and molecular properties of human astrocytes in acute hippocampal slices obtained from patients with temporal lobe epilepsy. Epilepsia. 2000; 41 Suppl 6:S181-4.
199. Schulte U, Thumfart JO, Klöcker N, Sailer CA, Bildl W, Biniossek M, Dehn D, Deller T, Eble S, Abbass K, Wangler T, Knaus HG, Fakler B. The epilepsy-linked Lgi1 protein assembles into presynaptic Kv1 channels and inhibits inactivation by Kvbeta1. Neuron. 2006 Mar 2; 49 (5) :697-706.
200. Seifert G, Carmignoto G, Steinhäuser C. Astrocyte dysfunction in epilepsy. Brain Res Rev. 2010 May; 63 (1-2) :212-21.
201. Seifert G, Schilling K, Steinhäuser C. Astrocyte dysfunction in neurological disorders: a molecular perspective. Nat Rev Neurosci. 2006 Mar; 7 (3) :194-206.
202. Semah F, Picot MC, Adam C, Broglin D, Arzimanoglou A, Bazin B, Cavalcanti D, Baulac M. Is the underlying cause of epilepsy a major prognostic factor for recurrence?. Neurology. 1998 Nov; 51 (5) :1256-62.
203. Senechal KR, Thaller C, Noebels JL. ADPEAF mutations reduce levels of secreted LGI1, a putative tumor suppressor protein linked to epilepsy. Hum Mol Genet. 2005 Jun 15; 14 (12) :1613-20.
204. Sheen SH, Kim JE, Ryu HJ, Yang Y, Choi KC, Kang TC. Decrease in dystrophin expression prior to disruption of brain-blood barrier within the rat piriform cortex following status epilepticus. Brain Res. 2011 Jan 19; 1369:173-83.
205. Soe R, Macaulay N, Klaerke DA. Modulation of Kir41 and Kir41-Kir51 channels by small changes in cell volume. Neurosci Lett. 2009 Jun 26; 457 (2) :80-4.
206. Sommer W: Erkrankung des Ammon´s Horn als ätiologisches Moment der Epilepsien. Arch Psychiatr Nurs 1880; 10:631-675
207. Sontheimer H. Voltage-dependent ion channels in glial cells. Glia. 1994 Jun; 11 (2) :156-72.

208. Spencer S, Spencer D. Surgery for epilepsy. Boston: Blackwell Scientific Publishers. 1991
209. Sperling MR, Lieb JP, Engel J Jr, Crandall PH. Prognostic significance of independent auras in temporal lobe seizures. Epilepsia. 1989 May-Jun; 30 (3) :322-31.
210. Stanhope JM, Brody JA, Brink E, Morris CE. Convulsions among the Chamorro people of Guam, Mariana islands II Febrile convulsions. Am J Epidemiol. 1972 Mar; 95 (3) :299-304.
211. Stefansson H, Rujescu D, Cichon S, Pietiläinen OP, Ingason A, Steinberg S, Fossdal R, Sigurdsson E, Sigmundsson T, Buizer-Voskamp JE, Hansen T, Jakobsen KD, Muglia P, Francks C, Matthews PM, Gylfason A, Halldorsson BV, Gudbjartsson D, Thorgeirsson TE, Sigurdsson A, Jonasdottir A, Jonasdottir A, Bjornsson A, Mattiasdottir S, Blondal T, Haraldsson M, Magnusdottir BB, Giegling I, Möller HJ, Hartmann A, Shianna KV, Ge D, Need AC, Crombie C, Fraser G, Walker N, Lonnqvist J, Suvisaari J, Tuulio-Henriksson A, Paunio T, Toulopoulou T, Bramon E, Di Forti M, Murray R, Ruggeri M, Vassos E, Tosato S, Walshe M, Li T, Vasilescu C, Mühleisen TW, Wang AG, Ullum H, Djurovic S, Melle I, Olesen J, Kiemeney LA, Franke B, GROUP, Sabatti C, Freimer NB, Gulcher JR, Thorsteinsdottir U, Kong A, Andreassen OA, Ophoff RA, Georgi A, Rietschel M, Werge T, Petursson H, Goldstein DB, Nöthen MM, Peltonen L, Collier DA, St Clair D, Stefansson K. Large recurrent microdeletions associated with schizophrenia. Nature. 2008 Sep 11; 455 (7210) :232-6.
212. Strohschein S, Hüttmann K, Gabriel S, Binder DK, Heinemann U, Steinhäuser C. Impact of aquaporin-4 channels on K+ buffering and gap junction coupling in the hippocampus. Glia. 2011 Jun; 59 (6) :973-80.
213. Stögmann E, Zimprich A, Baumgartner C, Aull-Watschinger S, Höllt V, Zimprich F. A functional polymorphism in the prodynorphin gene promotor is associated with temporal lobe epilepsy. Ann Neurol. 2002 Feb; 51 (2) :260-3.
214. Suga S, Suzuki K, Ihira M, Yoshikawa T, Kajita Y, Ozaki T, Iida K, Saito Y, Asano Y. Clinical characteristics of febrile convulsions during primary HHV-6 infection. Arch Dis Child. 2000 Jan; 82 (1) :62-6.
215. Sugawara T, Tsurubuchi Y, Agarwala KL, Ito M, Fukuma G, Mazaki-Miyazaki E, Nagafuji H, Noda M, Imoto K, Wada K, Mitsudome A, Kaneko S, Montal M, Nagata K, Hirose S, Yamakawa K. A missense mutation of the Na+ channel alpha II subunit gene Na(v)12 in a patient with febrile and afebrile seizures causes channel dysfunction. Proc Natl Acad Sci U S A. 2001 May 22; 98 (11) :6384-9.
216. Sutula T, He XX, Cavazos J, Scott G. Synaptic reorganization in the hippocampus induced by abnormal functional activity. Science. 1988 Mar 4; 239 (4844) :1147-50.
217. Takumi T, Ishii T, Horio Y, Morishige K, Takahashi N, Yamada M, Yamashita T, Kiyama H, Sohmiya K, Nakanishi S. A novel ATP-dependent inward rectifier potassium channel expressed predominantly in glial cells. J Biol Chem. 1995 Jul 7; 270 (27) :16339-46.
218. Tan NC, Mulley JC, Berkovic SF. Genetic association studies in epilepsy: "the truth is out there". Epilepsia. 2004 Nov; 45 (11) :1429-42.
219. Tang K, Fu DJ, Julien D, Braun A, Cantor CR, Köster H. Chip-based genotyping by mass spectrometry. Proc Natl Acad Sci U S A. 1999 Aug 31; 96 (18) :10016-20.

220. Tarkka R, Pääkkö E, Pyhtinen J, Uhari M, Rantala H. Febrile seizures and mesial temporal sclerosis: No association in a long-term follow-up study. Neurology. 2003 Jan 28; 60 (2) :215-8.
221. Theis M, Söhl G, Eiberger J, Willecke K. Emerging complexities in identity and function of glial connexins. Trends Neurosci. 2005 Apr; 28 (4) :188-95.
222. Theodore WH, Porter RJ, Penry JK. Complex partial seizures: clinical characteristics and differential diagnosis. Neurology. 1983 Sep; 33 (9) :1115-21.
223. Thrane AS, Rappold PM, Fujita T, Torres A, Bekar LK, Takano T, Peng W, Wang F, Thrane VR, Enger R, Haj-Yasein NN, Skare Ø, Holen T, Klungland A, Ottersen OP, Nedergaard M, Nagelhus EA. Critical role of aquaporin-4 (AQP4) in astrocytic Ca2+ signaling events elicited by cerebral edema. Proc Natl Acad Sci U S A. 2011 Jan 11; 108 (2) :846-51.
224. Tian GF, Azmi H, Takano T, Xu Q, Peng W, Lin J, Oberheim N, Lou N, Wang X, Zielke HR, Kang J, Nedergaard M. An astrocytic basis of epilepsy. Nat Med. 2005 Sep; 11 (9) :973-81.
225. Trysberg E, Blennow K, Zachrisson O, Tarkowski A. Intrathecal levels of matrix metalloproteinases in systemic lupus erythematosus with central nervous system engagement. Arthritis Res Ther. 2004; 6 (6) :R551-6.
226. Tsao CY, Mendell JR. Coexisting muscular dystrophies and epilepsy in children. J Child Neurol. 2006 Feb; 21 (2) :148-50.
227. Tsuboi T, Endo S. Genetic studies of febrile convulsions: analysis of twin and family data. Epilepsy Res Suppl. 1991; 4:119-28.
228. Tsuboi T. Epidemiology of febrile and afebrile convulsions in children in Japan. Neurology. 1984 Feb; 34 (2) :175-81.
229. Tsuboi T. Genetic aspects of febrile convulsions. Hum Genet. 1977 Sep 22; 38 (2) :169-73.
230. Umbricht D, Degreef G, Barr WB, Lieberman JA, Pollack S, Schaul N. Postictal and chronic psychoses in patients with temporal lobe epilepsy. Am J Psychiatry. 1995 Feb; 152 (2) :224-31.
231. Vadlamudi L, Andermann E, Lombroso CT, Schachter SC, Milne RL, Hopper JL, Andermann F, Berkovic SF. Epilepsy in twins: insights from unique historical data of William Lennox. Neurology. 2004 Apr 13; 62 (7) :1127-33.
232. Verkhratsky A, Steinhäuser C. Ion channels in glial cells. Brain Res Brain Res Rev. 2000 Apr; 32 (2-3) :380-412.
233. Vestergaard M, Pedersen CB, Sidenius P, Olsen J, Christensen J. The long-term risk of epilepsy after febrile seizures in susceptible subgroups. Am J Epidemiol. 2007 Apr 15; 165 (8) :911-8.
234. Vestergaard M, Basso O, Henriksen TB, Østergaard JR, Olsen J. Risk factors for febrile convulsions. Epidemiology. 2002 May; 13 (3) :282-7.
235. Villanueva V, Serratosa JM. Temporal lobe epilepsy: clinical semiology and age at onset. Epileptic Disord. 2005 Jun; 7 (2) :83-90.
236. Virchow, Rudolf Ludwig Karl, 1821-1902: Gesammelte Abhandlungen zur wissenschaftlichen Medicin / von Rudolf Virchow. -- 2. unveränderte Ausg. -- Hamm: C. Müller, 1862. -- xiv, 1024 p.
237. Volterra A, Meldolesi J. Astrocytes, from brain glue to communication elements: the revolution continues. Nat Rev Neurosci. 2005 Aug; 6 (8) :626-40.
238. Waite A, Tinsley CL, Locke M, Blake DJ. The neurobiology of the dystrophin-associated glycoprotein complex. Ann Med. 2009; 41 (5) :344-59.

239. Wallace RH, Wang DW, Singh R, Scheffer IE, George AL Jr, Phillips HA, Saar K, Reis A, Johnson EW, Sutherland GR, Berkovic SF, Mulley JC. Febrile seizures and generalized epilepsy associated with a mutation in the Na+-channel beta1 subunit gene SCN1B. Nat Genet. 1998 Aug; 19 (4) :366-70.
240. Wallace RH, Hodgson BL, Grinton BE, Gardiner RM, Robinson R, Rodriguez-Casero V, Sadleir L, Morgan J, Harkin LA, Dibbens LM, Yamamoto T, Andermann E, Mulley JC, Berkovic SF, Scheffer IE. Sodium channel alpha1-subunit mutations in severe myoclonic epilepsy of infancy and infantile spasms. Neurology. 2003 Sep 23; 61 (6) :765-9.
241. Wallraff A, Köhling R, Heinemann U, Theis M, Willecke K, Steinhäuser C. The impact of astrocytic gap junctional coupling on potassium buffering in the hippocampus. J Neurosci. 2006 May 17; 26 (20) :5438-47.
242. Walz R, Castro RM, Velasco TR, Alexandre V Jr, Lopes MH, Leite JP, Santos AC, Assirati JA Jr, Wichert-Ana L, Terra-Bustamante VC, Bianchin MM, Maciag PC, Ribeiro KB, Guarnieri R, Araújo D, Cabalero O, Moura R, Salim AC, Kindlmann K, Landemberger MC, Marques W Jr, Fernandes RM, Serafini LN, Machado HR, Carlotti CG Jr, Brentani RR, Sakamoto AC, Martins VR. Surgical outcome in mesial temporal sclerosis correlates with prion protein gene variant. Neurology. 2003 Nov 11; 61 (9) :1204-10.
243. Werb Z. ECM and cell surface proteolysis: regulating cellular ecology. Cell. 1997 Nov 14; 91 (4) :439-42.
244. Westerhuis J, Kourti I, MacGregor JF. Analysis of multiblock and hierarchical PCA and PLS models, *J. Chemometr.* **12** (1998), pp. 301–321.
245. Wieser HG, ILAE Commission on Neurosurgery of Epilepsy. ILAE Commission Report Mesial temporal lobe epilepsy with hippocampal sclerosis. Epilepsia. 2004 Jun; 45 (6) :695-714.
246. Wieshmann UC, Larkin D, Varma T, Eldridge P. Predictors of outcome after temporal lobectomy for refractory temporal lobe epilepsy. Acta Neurol Scand. 2008 Nov; 118 (5) :306-12.
247. Wilczynski GM, Konopacki FA, Wilczek E, Lasiecka Z, Gorlewicz A, Michaluk P, Wawrzyniak M, Malinowska M, Okulski P, Kolodziej LR, Konopka W, Duniec K, Mioduszewska B, Nikolaev E, Walczak A, Owczarek D, Gorecki DC, Zuschratter W, Ottersen OP, Kaczmarek L. Important role of matrix metalloproteinase 9 in epileptogenesis. J Cell Biol. 2008 Mar 10; 180 (5) :1021-35.
248. Winawer MR, Ottman R, Hauser WA, Pedley TA. Autosomal dominant partial epilepsy with auditory features: defining the phenotype. Neurology. 2000 Jun 13; 54 (11) :2173-6.
249. Yen DJ, Su MS, Yiu CH, Shih, Kwan SY, Tsai CP, Lin YY. Ictal speech manifestations in temporal lobe epilepsy: a video-EEG study. Epilepsia. 1996 Jan; 37 (1) :45-9.
250. Yoshida M, Suzuki A, Shimizu T, Ozawa E. Proteinase-sensitive sites on isolated rabbit dystrophin. J Biochem. 1992 Oct; 112 (4) :433-9.
251. Zarrelli MM, Beghi E, Rocca WA, Hauser WA. Incidence of epileptic syndromes in Rochester, Minnesota: 1980-1984. Epilepsia. 1999 Dec; 40 (12) :1708-14.
252. Zerhouni E. Medicine The NIH Roadmap. Science. 2003 Oct 3; 302 (5642) :63-72.
253. Zerhouni EA. Translational and clinical science--time for a new vision. N Engl J Med. 2005 Oct 13; 353 (15) :1621-3.

254. Zhang H, Verkman AS. Aquaporin-4 independent Kir41 K+ channel function in brain glial cells. Mol Cell Neurosci. 2008 Jan; 37 (1) :1-10. PubMed PMID:17869537;
255. Zlokovic BV. Remodeling after stroke. Nat Med. 2006 Apr; 12 (4) :390-1

Internet references

1. http://www.epigad.org
2. http://www.hapmap.org
3. http://www.ILAE.org
4. http://www.ncbi.nlm.nih.gov
5. http://www.sequenom.com
6. http://www.sanger.ac.uk/humgen/cnv
7. http://www.who.int

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