Epiglia

Genetic targets of epileptogenesis and pharmaco-resistance in brain glial cells; a translational research project on the genetic and molecular pathways of Temporal Lobe Epilepsy and Febrile Seizures
Epiglia – 4 collaborating centers

• Dep of Neurology, Oslo Univ Hospital – Rikshospitalet, Oslo, Norway  
  (Erik Taubøll, Kjell Heuser, Erlend Nagelhus)

• Institute of cellular Neurosciences, University of Bonn, Germany  
  (Group leader: prof Christian Steinhäuser)

• Kuopio Epilepsy Center, Univ of Eastern Finland, Finland  
  (Group leader: prof Reetta Kälviäinen)

• University Medical Center, Utrecht  
  (Group leader: Prof. Pierre de Graan)
Clinical background

• TLE represents a major health problem!
• Accounts for 1/3 of all epilepsy patients
• Chronic condition
• Several subgroups of TLE with MTLE-HS as the most severe
• Often pharmacoresistant
• Need for new treatment strategies!
Increasing interest in glia and epilepsy

Medline publications

Glia

Glia + epilepsy
Neuron-glia crosstalk

- Modification of synaptic transmission
- Electrotonic coupling through gap junctions
- Presynaptic neurons-postsynaptic glial cells
- Glia-glia transmission
- Gap-junction coupling – neuron-glia(?)
- Buffering of ions and water, glia-blood vessels

Bezzi and Volterra, 2001
Hippocampal sclerosis – highly epileptogenic glial tissue scar
Glial changes in hippocampal sclerosis

NORMAL

MTS
Epigia – hypothesis

Our hypothesis based on:

1. The important role for glia/astrocytes in regulation of brain excitability

2. The important role of genetics in different TLE subgroups and in FS

Main hypothesis:
Astrocytes play an important role in epileptogenesis and in generation, spread and maintenance of seizures in TLE and its different subgroups
Epiglia – main areas of research

• Genetic association studies based on human data and material from all centers, focusing on targets in brain glia (e.g. AQP4, Kir4.1, glutamine synthetase, connexin 30/43)

• MTLE-HS and FS mouse models

• Functional studies on human epileptic tissue
IP-1, Oslo

The group consist of:

• Epilepsy Research Group, Dep of Neurology, Oslo University Hospital – Rikshospitalet
• Centre for Molecular Medicine Norway and Nordic EMBL Partnership, Univ of Oslo
• Institute of Neurophysiology, Univ of Oslo
• Dep of Neurosurgery, Oslo University Hospital – Rikshospitalet
Methods available

• Mouse models of MTLE-HS. Will set up and use the intracortical kainic acid model developed by the Bonn group

• Slice preparations, neurophysiology

• Multiphoton laser scanning microscopy

• Surgical tissue from patients with MTLE-HS
Kir4.1 channel polymorphisms in TLE patients with childhood febrile seizures

Heuser et al. Epilepsy Res 2010;88:55-64
AQP4 enhances Ca$^{2+}$ signaling in astrocytes in experimental brain edema


Q: Will increased AQP4 expression in MTLE-HS contribute to Ca hyperactivity?
Work packages

• Study factors that promote epileptogenesis in a mouse model of MTLE-HS (i.c. kainic injection). Can these factors be modulated by novel AEDs or antiinflammatory drugs? Wild type and AQP4 (or other) KO mice will be used.

• Study Ca activity in astrocytes in sclerotic versus non-sclerotic hippocampi in patients with TLE

• Collaborate on association studies on glial targets in well defined phenotypes
Deciphering the impact of astroglial Kir4.1 dysfunction in MTLE
Methods

Patch-clamp in acute brain slices, isolated cells

Elektrophysiology

2P LSM

Single-cell sqRT-PCR

Conditional mutagenesis, transgenics (M. Theis)
### Transgenic mice, viral vectors

#### Hippocampus

<table>
<thead>
<tr>
<th>Type of transgene</th>
<th>Transgenic lines</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fluorescent indicator mice</strong></td>
<td>NG2kiEYFP, PLP-EGFP; NG2DsRed, Thy-1-YFP, Thy1-Syt1/ECFP, hGFAP-EGFP, Cx43kiECFP, Cx3CR1-EGFP, ALDH1L1-EGFP</td>
</tr>
<tr>
<td><strong>knockout</strong></td>
<td>DAOO loss of function; Pu1 KOs devoid of microglia; Kir4.1 KO; Cx30 KO; P2X7 KO; P2X4 KO, iNOS KO, CD11b KO, GNE KO; ASA KO; NAAGS-1 KO, NAAGS-2 KO, Cx47 KO; Ear2 KO, DBH KO; B7-H1 KO; PD-1 KO, TLR4 KO</td>
</tr>
<tr>
<td><strong>Cell-type specific knockout</strong></td>
<td>NG2-CreERT2, floxed <em>Kir4.1</em>; SRR KO (neuronal and glial); Astrocytic deletion of Asc-1; Cx43kiCreERT2, Cx30-CreERT2, floxed Ezrin, GFAP-cre, floxed Cx43; Cx43kiCreERT2, Cx30-CreERT2, floxed Cx30; floxed Cx30A88V, floxed Cx43G138R, Cx43K258Stop, floxed Cx43K378Stop; IDR related genes</td>
</tr>
<tr>
<td><strong>Cell-type specific overexpression</strong></td>
<td>Glial DAOO overexpression, G72 BAC transgenes, glial serine racemase overexpression; APP, APP/PS1 transgenes, astrocytic DN-IkappaBalpha; Abeta-BRI mouse, IDE related genes</td>
</tr>
<tr>
<td><strong>Cell-type specific transgene activation</strong></td>
<td>NG2-CreERT2, floxed channelrhodopsin-2; NG2kiCreERT2, Rosy, Z/EG;</td>
</tr>
<tr>
<td><strong>Tet System</strong></td>
<td>NG2-tTA, tetO-Lck-GCaMP2; GFAP-tTA, tetO-DN-Ezrin and tetO-activated-Ezrin; GFAP-tTA, tetOCPEB3-EGFP, tetO-DN-CPEB, tetO-Cx43, tetO-GS, tetO-GLT-1, tetO-AQ4, tetO-Dys</td>
</tr>
<tr>
<td><strong>Lentiviral transgenics</strong></td>
<td>Overexpression of miRNAs and miRNA inhibitors in astrocytes</td>
</tr>
</tbody>
</table>
K⁺ channel analysis in freshly isolated astrocytes

A

B1
10 μM Ba²⁺

B2
100 μM Ba²⁺

B3
1 mM Ba²⁺

Wash

A – B1

A – B2

A – B3

1 nA

20 ms

C

Ba²⁺-sensitive membrane conductance at -130 mV [%]

D

E

Seifert et al., J Neurosci, 2009
Astrocytes express Kir4.1
Unilateral intracortical kainate injection: an animal model of TLE

Time course of seizure activity after kainate treatment

Seizure frequency as a function of time after SE
Neuropathology of hippocampal sclerosis

Control/Lesion-ass. Epilepsy

Sclerosis

Questions:

- Considering lesion-tissue as a kind of ‘control’: Do astrocytes in sclerosis display modified properties that might be of relevance to epileptogenesis?

- Compare Kir4.1 expression in human MTLE-HS w/o antecedent febrile seizures

(Blümcke et al., 99)
Reduced glial Kir currents in HS

Hinterkeuser et al., 2000
Kir4.1 is downregulated in sclerotic human hippocampus
Work plan EuroEpinomics

WP1: Assess time course of altered Kir4.1 expression after SE in the KA mouse model of MTLE-HS
   - quantify Kir4.1 current densities and mRNA levels with post-recording single cell sqRT-PCR
     in the latent period, 3 months and 9 months post SE
   - Western blot analyses at the 3 time points
   - rescue with 17ß-oestradiol?

WP2: Determine impact of astroglial Kir4.1 dysfunction on seizure generation, mice with inducible deletion
   of Kir4.1 in astrocytes
   - compare K⁺ buffering in Cx43kiCreERT/++; Kir4.1fl/fl mice (SC stimulation, astrocyte recording w/o 0.1 mM Ba²⁺)
   - EEG recording / videomonitoring of Cx43kiCreERT/++; Kir4.1fl/fl mice
   - apply KA model Cx43kiCreERT/++; Kir4.1fl/fl mice (score of SE, duration of latent phase, frequency of spont. seizures
     in chronic phase, morphological changes, coupling)

WP3: Comparison of Kir4.1 expression in human MTLE-HS with and without antecedent FS
   - compare current densities in glial cells from TLE-FS and non TLE-FS patients; re-evaluate earlier data
   - comparison of Western blots, sqRT-PCR
IP-3, Kuopio
Group leader: prof Reetta Kälviäinen

Main topic:
Risk factors for temporal lobe epilepsy;

Gene association study of different temporal lobe epilepsy subtypes, especially those related with antecedent febrile seizures.
Why association studies in TLE?

- TLE denotes a location, not an entity
- TLE consists of several subgroups
- Different subgroups may have different causes and pathogenesis
- Response to treatment differs
- Different subgroups may need different treatment

Relation to FS
- TLE-FS may constitute a unique entity distinct from TLE without FS
- TLE and FS, common genetic basis?

Association analysis in TLE patients with/without FS
*From: Heuser et al. Epi Res, 2010*
Material

Will use patient and control materials from all 4 sites.

Altogether:
• >1000 TLE patients + controls extensively phenotyped
• DNA/RNA from most patients
• Brain tissue, > 500 patients
Work plan, Kuopio

WP1. Integration of the phenotypic data from all four cohorts into a common database.

WP2. Extraction of DNA from blood samples

WP3. Association studies, focus on targets in glia as AQP4, Kir4.1, others according to further discussions
AP-1, Utrecht
Group leader: prof. Pierre de Graan

Febrile seizures and epileptogenesis in mice and men

University Medical Center Utrecht, Utrecht, Holland
Epilepsy UMCU

Dutch center for childhood epilepsy

child neurology

neurophysiology

neurosurgery

neuroscience & pharmacology

medical genetics

Animal models for febrile seizures and TLE

50 TLE surgeries/year

Dutch referral center for childhood epilepsy
Mouse Febrile Seizure model

Mouse pups (p14) are exposed to a warm-air stream to induce experimental febrile seizures.

Febrile seizure susceptibility is defined as the latency to tonic-clonic convulsions.
Forward genetics:
Chromosome substitution strains (CSS)

- Donor and host differ in FS susceptibility
- Substitution strains with donor phenotype carry QTL for FS

Singer et al., 2004
Febrile seizures susceptibility of mouse strains in the CSS panel

* significant difference compared to C57BL/6J p<0.003

Identification of 6 chromosomes carrying FS susceptibility genes.

Hessel et al., GBB (2009)
Workpackages - AP Utrecht

• Mapping febrile seizure QTLs on mouse chromosome 1 and 2

• Candidate gene selection (bioinformatics)

• Candidate gene identification

• Functional interference studies in mice

• Sequencing of candidate gene in human TLE patients with and without FS
Epiglia – overall collaboration

- Individual projects in each site focusing on glial mechanisms in epileptogenesis in TLE and FS
- Joint projects. 1) human association studies, 2) collaboration on mouse models on TLE and FS.

Main focus:
1. Bonn: Study the impact of Kir4.1 on epileptogenesis and seizure activity in a mouse model of TLE (i.c. kainic acid), and in human tissue
2. Utrecht: Focus on FS, and FS as a possible origin of TLE. Mice and humans
3. Kuopio: Focus on human genetic association studies
4. Oslo: Coordinative role. Will establish the i.c. kainic acid mouse model for studies on epileptogenesis, participate in association studies
Thank you!