Progressive Myoclonus Epilepsies – on the way to precision medicine?

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Folkhälsan Institute of Genetics, Helsinki
Outline

• Definition and evolution of molecular genetic understanding of progressive myoclonus epilepsies
• Deciphering the molecular basis of unsolved cases with next generation sequencing technologies – identification of a new PME syndrome
• Towards precision medicine in PMEs – disease mechanisms in Unverricht-Lundborg disease (EPM1)
• Conclusion
Progressive Myoclonus Epilepsies

PMEs

- Heterogeneous group of rare genetic epilepsies
- Overall prevalence ~ 1:100,000
  - regional variation due to founder effects, e.g. in Finland
    prevalence 1:25 000
Progressive Myoclonus Epilepsies
PMEs

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- Myoclonic seizures; action myoclonus
- Tonic-clonic seizures
- Progressive neurological decline
  - Ataxia
  - ± Cognitive decline
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Many diseases, often autosomal recessively inherited
Diagnosis challenging, poor prognosis, no cure
Unverricht-Lundborg disease (ULD / EPM1): clinical features

- autosomal recessive neurodegenerative disorder
- age of onset: 6-18 years
- action-activated progressive myoclonus; stimulus-sensitivity
- tonic-clonic seizures
- ataxia
- no major cognitive decline
- wide variability in the clinical presentation
- valproic acid; clonazepam piracetam, levetiracetam, topiramate; zonisamide; brivaracetam
- underlying gene: cystatin B / stefin B
Evolution of gene identification

Functional cloning

- Before 1990
- Knowledge on the biochemical basis of the disease utilized
- *F8* in Hem A;
  *PAH* in PKU
- *GBA* in Gaucher disease
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  - Several families with homogenous phenotype / single large pedigrees
  - Major clinical PME forms
  - Novel rare clinical PME forms

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- **2010 -**
  - Massive parallel sequencing of exomes/genomes
  - Gene identification possible even in single families
  - Novel very rare PME forms
  - PMEs with atypical presentation
  - Novel “common” PMEs escaping detection by linkage

- **Functional cloning**
- **Positional cloning / gene identification**
- **Next generation sequencing**
# PME genes (EPM designation in OMIM)

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<th>Inheritance pattern</th>
<th>Gene(s)</th>
<th>Protein function/molecular pathway</th>
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<tr>
<td>Unverricht-Lundborg disease (EPM1)</td>
<td>AR</td>
<td><strong>CSTB</strong></td>
<td>Inhibitor of lysosomal cysteine proteases</td>
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<td>Lafora disease (EPM2A/B)</td>
<td>AR</td>
<td><strong>EPM2A, NHLRC1</strong></td>
<td>A phosphatase and a ubiquitin ligase involved in glycogen metabolism</td>
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<td>PME type 3 (EPM3)</td>
<td>AR</td>
<td><strong>KCTD7</strong></td>
<td>Modulation of potassium ion channel function (?)</td>
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<td>PME type 8 (EPM8) (one family)</td>
<td>AR</td>
<td><strong>CERS1</strong></td>
<td>Ceramide synthase -1</td>
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<td>PME type 9 (EPM9) (one family)</td>
<td>AR</td>
<td><strong>LMNB2</strong></td>
<td>Nuclear lamin protein</td>
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## PME genes: neuronal ceroid lipofuscinoses

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<tr>
<td>Infantile, CLN1</td>
<td>AR</td>
<td>PPT1</td>
<td>Lysosomal palmitoyl-protein thioesterase</td>
</tr>
<tr>
<td>Juvenile, CLN3</td>
<td>AR</td>
<td>CLN3</td>
<td>Lysosomal membrane protein of unknown function</td>
</tr>
<tr>
<td>Late infantile, classical, CLN2</td>
<td>AR</td>
<td>TPP1</td>
<td>Lysosomal tripeptidyl peptidase</td>
</tr>
<tr>
<td>Variant late infantile, CLN5</td>
<td>AR</td>
<td>CLN5</td>
<td>Lysosomal soluble protein of unknown function</td>
</tr>
<tr>
<td>Northern epilepsy (Finland); Variant late infantile NCL, CLN8</td>
<td>AR</td>
<td>CLN8</td>
<td>ER membrane protein of unknown function</td>
</tr>
<tr>
<td>Variant late infantile, CLN6</td>
<td>AR</td>
<td>CLN6</td>
<td>ER membrane protein of unknown function</td>
</tr>
<tr>
<td>Congenital, CLN10</td>
<td>AR</td>
<td>CTSD</td>
<td>Lysosomal aspartatyl protease</td>
</tr>
<tr>
<td>Variant late infantile, CLN7</td>
<td>AR</td>
<td>MFSDB</td>
<td>Lysosomal membrane protein, transporter</td>
</tr>
<tr>
<td>Adult, CLN4</td>
<td>AD</td>
<td>DNAJC5</td>
<td>Synaptic vesicles</td>
</tr>
<tr>
<td>Adult, CLN11 (one family)</td>
<td>AR</td>
<td>GRN</td>
<td>Secretory pathway, an autocrine growth factor</td>
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<tr>
<td>Juvenile, CLN12 (one family)</td>
<td>AR</td>
<td>ATP13A2</td>
<td>Lysosomal membrane, ATPase, transporter</td>
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<td>Adult, CLN13</td>
<td>AR</td>
<td>CTSF</td>
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# PME genes: other

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<tr>
<td>Gaucher disease</td>
<td>AR</td>
<td>GBA</td>
<td>Lysosomal beta-glucocerebrosidase</td>
</tr>
<tr>
<td>MERRF</td>
<td>mt</td>
<td>MTTK</td>
<td>tRNA for lysine</td>
</tr>
<tr>
<td>Sialidosis, types 1 and 2</td>
<td>AR</td>
<td>NEU1, GSL</td>
<td>Lysosomal neuraminidase, breaks down oligosaccharides</td>
</tr>
<tr>
<td>DRPLA</td>
<td>AD</td>
<td>ATN1</td>
<td>Accumulation of mutated ATN1 in nucleus -- &gt; transcriptional dysregulation</td>
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<tr>
<td>Neuroaxonal Dystrophy</td>
<td>AR</td>
<td>PLA2G6</td>
<td>Phospholipase A6, calcium independent</td>
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<td>SMAPME</td>
<td>AR</td>
<td>ASAH1</td>
<td>Lysosomal acid ceramidase; degradation/synthesis of ceramide</td>
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Very rare causes:
- Juvenile Huntington disease (*HTT*)
- Atypical inclusion body disease (*SERPIN1*)
- Alzheimer disease (very early onset)
- Senile myoclonic epilepsy (Down syndrome)
- etc.
Diagnostic yield in large PME series

- genetic causes of PME among 204 patients in Italy

- a nationwide multicenter study; 25 centers

- Franceschetti et al. Neurology 2014
Unsolved PME Cases: Phase 1 Whole Exome Sequencing (WES)

• 84 unrelated PME patients without a genetic diagnosis
  – internationally collected in over a 20-year period; mostly Europeans, ~50% Italian; all negative for mutations in CSTB
  – Sam Berkovic, Melbourne

  – 70 were sporadic
    11 had affected siblings or cousins
    3 had affected children or an affected parent
    • recessive, dominant/de novo, mitochondrial inheritance models

  – Sanger Institute, UK
Variant analysis strategy

Exomes of 84 unrelated PME patients
- Avg. 36528 variants per patient

Variant filtering - step 1
- potentially damaging coding variants in CCDS genes
- Avg. 1391 variants per patient

Recessive model
- Variant filtering - step 2
  - homozygous, hemizygous, or potentially compound heterozygous variants (<1% allele frequency)
  - Avg. 4.5 genes per patient
  - Known disease genes
  - Potential novel PME genes

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Muona et al. Nat Genet 2015
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In addition: mitochondrial inheritance model

Muona et al. Nat Genet 2015
Unsolved PME Cases: Phase 1 WES

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- Surprise 1: The majority had a dominantly inherited or a de novo mutation
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- 1/25 did not have PME \((SCN1A)\)
- Surprise 1: The majority had a dominantly inherited or a \textit{de novo} mutation
- Surprise 2: 11/26 had mutations in a new gene

Muona et al. Nat Genet 2015
A recurrent missense mutation in *KCNC1* is a new cause of PME

- Exactly the same, recurrent missense mutation in 11/84 patients: c.959G>A; p.R320H
- Mutation pathogenic in the heterozygous state
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- Encodes a subunit (Kv3.1) of the Kv3 subfamily of voltage-gated tetrameric potassium ion channel
- AD or *de novo* mutations in *KCNC3* (Kv3.3) cause SCA

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- AD or \textit{de novo} mutations in \textit{KCNC3} (Kv3.3) cause SCA
- Kv3.1 is predominantly expressed in neurons that fire action potentials at high frequency
  - inhibitory GABAergic interneurons of neocortex and hippocampus, cerebellar granule neurons

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Muona et al. Nat Genet 2015
Arg320His has a dominant-negative loss-of-function effect on Kv3.1 K+ channel function

![Graphs showing current amplitudes for WT and Arg320His channels.](image)

Mutant channel expressed alone produces no K+ currents

Muona et al. Nat Genet 2015
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WT+mutant shows 80% reduction in potassium currents (dominant-negative loss of function)

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WT+mutant activates at more negative voltages (gain of function)

Muona et al. Nat Genet 2015
The Arg320His mutation in \textit{KCNC1} defines a new PME syndrome: **MEAK**

- Myoclonus Epilepsy and Ataxia due to $K^+$ channel mutation
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  - 16 from original study (Muona et al, 2015)
  - 4 subsequently identified
  - majority sporadic cases; two families

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MEAK has a clinical presentation similar to Unverricht-Lundborg disease

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<th>Classical ULD</th>
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<td>First symptom</td>
<td>Myoclonus (or “tremor”)</td>
<td>GTCS or myoclonus</td>
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<td>Age onset: mean</td>
<td>10 years</td>
<td>11 years</td>
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<td>Age onset: range</td>
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<td>Progressive features</td>
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<td>Cognitive decline</td>
<td>Mild or absent</td>
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<td>Mode of inheritance</td>
<td>Autosomal dominant</td>
<td>Autosomal recessive</td>
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<tr>
<td>Gene</td>
<td>KCNC1</td>
<td>CSTB</td>
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<td>Protein function</td>
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Potential therapy for *KCNC1* associated PME?
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- UK-based biotech company Autifony Therapeutics Ltd. has recently developed compounds activating Kv3 channels.
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Potential therapy for *KCNC1* associated PME?

- UK-based biotech company Autifony Therapeutics Ltd. has recently developed compounds activating Kv33 channels.
- Autifony compounds are tested in cellular and animal models to study the effect of these molecules on the Arg320His mutant Kv3 function.
- A genetically homogeneous, clinically well-characterized patient population will enable fast translation of these preclinical studies to clinical phase.
PME genetics – before and after WES phase 1

Before:
- Known causes
- Unknown cause

After:
- Known causes
- Unknown cause
  - KCNC1
  - SACS
  - TBC1D24
  - PRNP

Previously known PME genes
PME genetics – before and after WES phase 1

BEFORE

- Known causes
- Unknown cause

AFTER

- Known causes
- Unknown cause
- KCNC1
- SACS
- TBC1D24
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- Previously known PME genes

• PME WES phase 2 ongoing; trio design, 37 unrelated cases
Molecular genetic diagnosis of PMEs

- Challenge: clinical and genetic heterogeneity
  - Next Generations Sequencing panels
  - Not all PME genes covered in available panels
Molecular genetic diagnosis of PMEs

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• Dodecamer expansion mutation in late-childhood/adolescent onset PMEs without cognitive decline
  → can not be detected in current panels!
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- WES
  - Relatively fast, cost-effective
  - Interpretation more challenging – not yet in wide clinical use
  - Possibility to identify mutation in “unexpected” genes
EPM1: loss-of function mutations in the CSTB gene

- dodecamer repeat expansion in the CSTB promoter
  - 90% of disease alleles
  - number of repeats >30

- 16 other mutations reported

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- patients compound heterozygous for the dodecamer expansion and p.Arg68* have a more severe phenotype
  - earlier age of onset
  - more severe myoclonus
  - drug-resistant TC seizures
  - lower cognitive performance

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  - lower cognitive performance
  - mutant protein degraded?

Homozygous stop/fs mutations in \textit{CSTB} associated with a neonatal onset severe encephalopathy

  - progressive microcephaly, early severe developmental delay and severe dyskinesia in two siblings
  - MRI: delayed myelination

  - profound global developmental delay, microcephaly, cortical blindness, axial hypotonia with appendicular hypertonia, dysmorphia in two siblings
  - MRI: diffuse hypomyelination with progressive loss of myelin signal
EPM1: CSTB protein

- cystatin B; stefin B; a 98 aa/11kDa; inhibitor of cysteine cathepsins (B, H, L, S, K, F)
- ubiquitous tissue expression
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- ubiquitous tissue expression
- cytoplasmic and nuclear localization, partial co-localization with lysosomal marker proteins
  - missense mutant proteins fail to associate with lysosomes
EPM1: CSTB protein

- cystatin B; stefin B; a 98 aa/11kDa; inhibitor of cysteine cathepsins (B, H, L, S, K, F)
- ubiquitous tissue expression
- cytoplasmic and nuclear localization, partial co-localization with lysosomal marker proteins
  - missense mutant proteins fail to associate with lysosomes
  - upon inflammatory stimuli may translocate to mitochondria (Maher et al. 2014)
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Alakurtti et al. Eur J Hum Genet 2005
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  - CSTB interacts with histones H2A.Z, H2B and H3, as well as with cathepsin L (Ceru et al. 2010)
- significantly reduced CSTB expression and inhibitory activity; increased cathepsin activity in EPM1 patients

Alakurtti et al. Eur J Hum Genet 2005
Cstb\(^{-/-}\) mouse model for EPM1

- myoclonic seizures (1 mo)
- progressive ataxia (6 mo)

Pennacchio et al. Nature Genet 1998
Cystatin B deficiency sensitizes neurons to oxidative stress

Lehtinen et al., J Neurosci 2009
Cystatin B deficiency sensitizes neurons to oxidative stress

Lehtinen et al., J Neurosci 2009
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Lehtinen et al., J Neurosci 2009
Cathepsin B mediates oxidative stress-induced neuronal death downstream of Cystatin B

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Cystatin B deficiency disrupts cerebellar redox homeostasis

6 mo

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6 mo
Disease progression in the Cstb⁻/⁻ mouse model for EPM1

- P14
  - Myoclonus
- 1 mo
- 2 mo
- 4 mo
- 6 mo
  - Ataxia

Disease progression in the \textit{Cstb}^{-/-} mouse model for EPM1

Disease progression in the $Cstb^{-/-}$ mouse model for EPM1

- Microgliosis
- Astrogliosis

1 mo $Cstb^{-/-}$

- P14
- 1 mo
- 2 mo
- 4 mo
- 6 mo

Myoclonus

Ataxia

Disease progression in the \textit{Cstb^{-/-}} mouse model for EPM1

- Microgliosis
- Astrogliosis
- Neuron loss

![Graph showing disease progression]

- Estimated neuron number vs. age
- Myoclonus at P14
- Ataxia at 6 mo

Disease progression in the Cstb\(^{-/-}\) mouse model for EPM1

- Microglossis
- Astroglossis
- Neuron loss
- White matter damage

6 mo
Cstb\(^{-/-}\)

P14 1 mo 2 mo 4 mo 6 mo
Myoclonus
Ataxia

Disease progression in the Cstb−/− mouse model for EPM1

- Microgliosis
- Astrogliosis
- Neuron loss
- White matter damage
- Volume loss in cortex and CB

Timeline:
- P14
- 1 mo
- 2 mo
- 4 mo
- 6 mo

Myoclonus
Ataxia

Disease progression in the Cstb-/- mouse model for EPM1

- Microgliosis
- Astrogliosis
- Neuron loss
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Key:
- P14
- 1 mo
- 2 mo
- 4 mo
- 6 mo

- Myoclonus
- Ataxia

Early microgliosis in Cstb-/- mice

F4/80

A: P14
Cstb-/-

B: P14
Cstb-/-

C: 1 mo
Cstb-/-

D: 4 mo

E: 6 mo

F: 6 mo

H

P14 ctrl
P14 Cstb-/-

1 mo Cstb-/-

2 mo Cstb-/-

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6 mo Cstb-/-
Altered functional properties of \( \text{Cstb}^{-/-} \) microglia \textit{in vitro}
Altered functional properties of Cstb\(^{-/-}\) microglia \textit{in vitro}\n
\textbf{Cstb} expression in CNS cells

\textbf{Chemokine release}

\textbf{Chemotaxis}

Okuneva, Körber et al. Glia 2015
Altered functional properties of \textit{Cstb}^{-/-} microglia \textit{in vitro}

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\textbf{Phagocytosis}

Okuneva, Körber \textit{et al.} \textit{Glia} 2015
Altered functional properties of Cstb−/− microglia in vitro

Cstb expression in CNS cells

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Chemotaxis

- Impaired signaling via the interferon pathway

Okuneva, Körber et al. Glia 2015
Increased apoptosis and compromised phagocytosis in hippocampus of P14 Cstb<sup>−/−</sup> mice

Sierra et al., unpublished
Cstb<sup>−/−</sup> mouse microglia are polarized towards the pro-inflammatory phenotype

M1: pro-inflammatory phenotype
M2: anti-inflammatory phenotype

Okuneva et al. unpublished
Increased expression of inflammatory markers in $Cstb^{-/-}$ mouse cortex

Okuneva, Körber et al., Glia 2015
Neuroinflammation in Cstb<sup>-/-</sup> mouse brains

- Expression of immune and defense response genes is upregulated
- The number of CD3<sup>+</sup> T-lymphocytes and LY-6B.2<sup>+</sup> granulocytes ↑
- The amount of CD45<sup>++</sup>F4/80<sup>+</sup> macrophages ↑
- Vascularization ↑
- BBB intact

Elevated pro-inflammatory cytokine levels in \textit{Cstb}^{-/-} mouse serum

Elevated pro-inflammatory cytokine levels in Cstb\(^{-/-}\) mouse serum

Cstb\(^{-/-}\) mice are more sensitive to LPS-induced lethal endotoxemia
LPS induces secretion of pro-inflammatory cytokines in serum of Cstb−/− mice

Altered inflammasome and IL-1\(\beta\) pathway activation in Cstb\(^{-/-}\) macrophages

Is EPM1 an autoinflammatory disease?
Alterations in GABAergic signaling in cerebellum of pre-symptomatic Cstb⁻/⁻ mice

- A shift of the balance towards decreased inhibition
Alterations in GABAergic signaling in cerebellum of pre-symptomatic Cstb\(^{-/-}\) mice

- a shift of the balance towards decreased inhibition
- no significant difference in the number of interneurons
- diminished number of GABAergic terminals
- reduced ligand binding to GABA\(_A\) receptors

Tegelberg, Joensuu et al. PLoS ONE 2014
Hypothesis
Hypothesis
Hypothesis

Altered functional properties, inflammatory mediators
Hypothesis

Altered functional properties, inflammatory mediators
Hypothesis

Altered functional properties, inflammatory mediators

CSTB

MG

Altered neuronal excitability

CSTB

N
Hypothesis

Altered functional properties, inflammatory mediators

CSTB

Altered neuronal network / excitability

MG

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CSTB

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CSTB

Altered functional properties, inflammatory mediators

Activation of microglia

Altered neuronal network / excitability

Altered neuronal excitability
Hypothesis

CSTB

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Activation of microglia

Pro-inflammatory mediators

Altered neuronal network / excitability

N

CSTB

Altered neuronal excitability
Hypothesis

CSTB

Altered functional properties, inflammatory mediators

Activation of microglia

Pro-inflammatory mediators

Altered neuronal network / excitability

Neuronal damage / death

CSTB

Altered neuronal excitability
Hypothesis

CSTB → Altered functional properties, inflammatory mediators

Activation of microglia → ATP, other inflammatory triggers → Pro-inflammatory mediators → Altered neuronal network / excitability → Neuronal damage / death → Neuronal excitability → CSTB
CSTB

Altered functional properties, inflammatory mediators

Activation of microglia

ATP, other inflammatory triggers

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CSTB

Altered neuronal excitability
Unifying pathophysiological mechanisms? EPM1 and MEAK

• very similar clinical and neurophysiological features
• different molecular genetic bases

• Kv3.1 is predominantly expressed in neurons that fire action potentials at high frequency
  • inhibitory GABAergic interneurons of neocortex and hippocampus, cerebellar granule neurons
  • p.Arg320His likely to cause disinhibition due to the impaired firing of fast-spiking GABAergic interneurons

• evidence for alterations in GABAergic signalling in young Cstb⁻/⁻ mice
• evidence for impaired/altered cortical inhibitory mechanisms in EPM1 patients
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• loss of GABAergic inhibition the final common pathway?
Hopes for Precision Medicine Approaches

- Essential that the field moves from diagnosis to translation
Hopes for Precision Medicine Approaches

• Essential that the field moves from diagnosis to translation
• Modulation of a specific neurophysiological system?
Hopes for Precision Medicine Approaches

• Essential that the field moves from diagnosis to translation
• Modulation of a specific neurophysiological system?
• Therapies directed at molecular lesions
  – Ion channels may be most tractable (e.g. MEAK)
  – Modulation of inflammation / oxidative stress (e.g. EPM1/ULD)
    • timing of treatment?
  – Downstream targets of other genes
Conclusions
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• PME is a clinically and genetically highly heterogeneous group of diseases
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- Specific diagnosis allows determination of prognosis, rational genetic counselling, possible prenatal diagnosis and treatment
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Conclusions

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• Focus now should be on mechanisms and translational therapeutic approaches
  
  – EPM1: mechanisms involve a complex interplay between neurons and glia affecting neuronal survival and involving oxidative stress and inflammation
# Acknowledgements

**LEHESJOKI GROUP: PME**

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