Developing genetic therapies for neurological disorders

Cobb lab
Institute of Neuroscience and Psychology
Centre for Neuroscience

Abnormalities in brain development:

Differentiation / migration  |  Axon path-finding  |  Cellular complexity  |  Synapse formation / maturation
Rett Syndrome & X-linked ID

- Lifelong intellectual & motor disability, epilepsy, breathing dysfunction
- X-linked genes: 
  - MECP2
    - Reader of epigenetic marks / regulates gene expression
  - CDKL5
    - Role in gene regulation / synaptic plasticity

Timeline:
- Birth
- ~1-2 years: Normal development
- 5+ years: Stagnation, Regression, Stationary, Motor deterioration
Rett syndrome can be accurately modeled in mice

- Mouse model created by KO of *Mecp2* gene

  - Normal early development ✓
  - Cognitive impairment ✓
  - Gait disturbances ✓
  - Breathing dysfunction ✓
  - Seizures / aberrant EEG ✓
  - Limb clasping ✓
  - Early male death ✓
Reversal in a range of behavioural/motor tasks

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### Improvement

- **Tremor**: ✓
- **Locomotion**: ✓
- **Exploratory activity**: ✓
- **Balance beam**: ✓
- **Grip strength**: ✓
- **Rotarod**: ✓
- **Gait**: ×
- **Breathing**: ✓
- **Cognition**: ?
- **Structural remodelling**: ✓
- **Behaviour (anxiety)**: ✓
- **Epileptiform activity**: ✓
- **Thermoregulation**: ✓
- **Abnormal EEG**: ✓

Robinson et al. 2012 Brain. PMID: 22525157.
Gene therapy - conventional gene augmentation therapy

2013

Improved Survival and Reduced Phenotypic Severity Following AAV9/MECP2 Gene Transfer to Neonatal and Juvenile Male MeCP2 Knockout Mice

Kamal KE Gadalla, Mark ES Bailey, Rosemary C Spike, Paul D Ross, Kenton T Woodard, Sahana Nagabhushan Kalburgi, Lavanya Bachaboina, Jie V Deng, Anne E West, R Jude Samulski, Steven J Gray and Stuart R Cobb

Smarter cassettes with regulatory elements

Tuneable (pharmacologically regulated) expression systems

Capsids that target / de-target particular cells
Looking to the future: mutation repair in the brain - editing, skipping, inclusion, read-through, trans splicing...

**MeCP2**

- Human mutation Knock-in
- Knock-in
- MeCP2-GFP
- DAPI

**Mutation repair**

- DNA binding domain recognising T158M allele
- FokI nuclease

**CDKL5**

- Brain-dominant CDKL5 isoform
- Distribution of pathogenic mutations
- Prototypical mutations to be modelled
- Strategies for personalised medicine
  - Splice correction therapy
  - Exon inclusion
  - Splice modulation therapy
  - Exon skipping
  - Ribosomal read-through
  - NMD modulation
  - Exon skipping

- UTR1
- UTR2
- CDKL5_m1
- CDKL5_m2
- CDKL5_m3
- GAPDH
- Whole Brain
- Synaptic membrane
- + + + + + RT

- ATG
- 1 1a 1b 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 16b 17 18 19 20 21
- 3'UTR
What we need now…

Expertise in cell biology generally / primary neuronal culture / neuronal stem cells

Expertise in patient derived cells (fibroblasts, iPS etc)

Expertise in rodent EEG / telemetry

Better animal facilities for behavioural testing / cognitive studies

Mitochondrial imaging
Collaborations

Cobb Lab
Kamal Gadalla
Ralph Hector
Elaine Hunter
Paul Ross
Thishnapha Vudhironarit
Daniela Minchelia
John Craig
Noha Bahey

Edinburgh
Adrian Bird / Jacky Guy / Jim Selfridge
Peter Kind

Aberdeen
Gernot Riedel

Trinity College Dublin
Kumlesh Dev

Glasgow
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Adam/Katherine West (Cancer)
Liz Tanner (Engineering)
John Riddell (INP)
Julia Edgar (III)

North Carolina
Steve Gray

Vollum Institute & HHMI
Gail Mandel

University of Milan
Nicoletta Landsberger
Charlotte Kipstrup-Nielsen

Stirling
Bruce Graham

Strathclyde
Trevor Bushell (SIBS)
Michele Zagnoni (Engineering)