Canine Degenerative Myelopathy (DM): A relevant animal model of familial amyotrophic lateral sclerosis?

Aim 1: Identify disease mechanism?
Aim 2: Identify useful biomarkers?

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DM is a neurodegenerative conditions affecting the spinal cord of adult/aged dogs

- A chronic progressive disease first described in 1973 (Averill, 1973)
- Various terms
  - Chronic degenerative radiculomyelopathy (CDRM) (Griffiths and Duncan, 1975)
  - German Shepherd dog myelopathy (GSDM)
  - Other breeds also affected
- Syndrome of progressive pelvic limb ataxia and weakness
  - Chronic UMN disease
- Pathology: (Johnston et al 2000)
  - Degeneration of motor and sensory white matter tracts,
- Genetics (Awano et al 2006)
  - Significant finding is an association with a mutation in the Sod1 gene

![Diagram showing the stages of DM progression]

- Disease Onset 0–6 months:
  - Scuffing marks in two middle toes in one or both pelvic limbs
- Early Stage 6–9 months:
  - Asymmetrical pelvic limb ataxia with UMN signs
  - Flaccid paralysis and development of mild to moderate muscle atrophy (LMN signs) with/without urinary and faecal incontinence
- Advanced Stage 14–24 months:
  - UMN signs in thoracic limbs progresses to LMN paralysis
  - Generalised muscle atrophy and tetraplegia
  - Brain stem signs (dysphagia and inability to bark)
- End Stage ≥ 36 months:
  - Tentative cause of death is due to paralysis of intercostal muscles leading to respiratory failure
Methods Employed: investigation of disease mechanism

• Archive tissue bank from post mortem material
  – Pamela Johnston et al 1994-99
  – fixed and fresh frozen CNS

• Fixed material
  – Spinal cord and brain suitable for IHC studies
  – Perfusion fixed suitable for EM

• Fresh frozen material stored in LN
  – Suitable for protein expression and mRNA studies

• In vitro studies
  – Expression of tagged (GFP/Cherry) wildtype and mutant SOD1 protein
  – Neuroblastoma and motor neuron derived cells
  – SOD1 activity gels
Methods employed: biomarkers identification

• Establish working interaction between research and clinical staff
  – Geographical issues
  – Dedicated staff (Dr Intan Shafie PhD)
  – Sample bank (Julien Guevar)

• Sample collection and case history (CSF, blood and urine)
  – Optimised protocols for collection and storage
  – In house genotyping protocol
  – Access to case history

• Analysis
  – Follow the fALS field (classic veterinary approach)
  – Gel based protein profile assessment (precipitation required, exosome isolation)
  – Mass spec analysis of whole proteome
  – Validation studies (species specificity etc)
Disease mechanisms: pathology and biochemistry

• Pathology: Dr Livia Henderson (resident)
  – Assess SOD1 aggregate accumulation
  – Assess its relationship with neuronal/glial integrity
  – Non cell autonomous?

• Biochemical studies: Yao Qi (masters)
Disease mechanisms: *In vitro* studies (Yao Qi)

- Mutant SOD1 forms aggregates
  - Activity gel suggests function may be retained
  - Association with mitochondria not dramatically altered
  - Possible activation of autophagy

**Mimic heterozygote carriers**
- WT and mutant SOD1 interact

**Image:**
- pEGFP-Sod1\(^\text{WT}\)
- pEGFP-Sod1\(^\text{mutant}\)

**AUTOPHAGY**
- SOD1
- WT SOD1
- Mutant SOD1

**MITOTRACKER**
- WT
- DM
CSF Biomarker identification: is one enough?

- **Clusterin** (apolipoprotein J)
  - A potential candidate for DM
  - But elevated in IVDD
  - Does not fulfil biomarker criteria
  - May inform on disease mechanisms

- **Other candidates**
  - TTR and cystatin C

- **A panel of biomarkers are required**
Collaborators

• **Infrastructure**
  – Facilities available at Garscube (confocal, proteomics, etc)

• **Clinical neurology team (SAH)**
  – Source of material (CSF, blood and urine)

• **Proteomics (CSF and urine)**
  – Richard Burchmore (protein ID)
  – William Mullen (multiple candidates-CE MS)

• **Human material**
  – Martin Turner (Oxford)

• **In vitro material**
  – Adrian Higginbottom, University of Sheffield (cells and tissue)
  – Conformational sensitive antibodies (industrial collaboration)
How to progress?

- **Expertise**
  - Up to date developments-MND community
  - Understanding motor pathways and what to look for in DM
  - What are the most informative markers?

- **Research support**
  - Need continuity of research with dedicated student (PhD)
  - Funding (Vet school has been supportive but financially limited)

- **Additional cases**
  - Need to initiate a programme to attract more cases
  - What can we offer clients? MRI?
  - Co-ordinate euthanasia with PM (cost)
  - Ethical approval

- **DM: new model old problems**