University of Glasgow | College of Medical, Veterinary & Life Sciences

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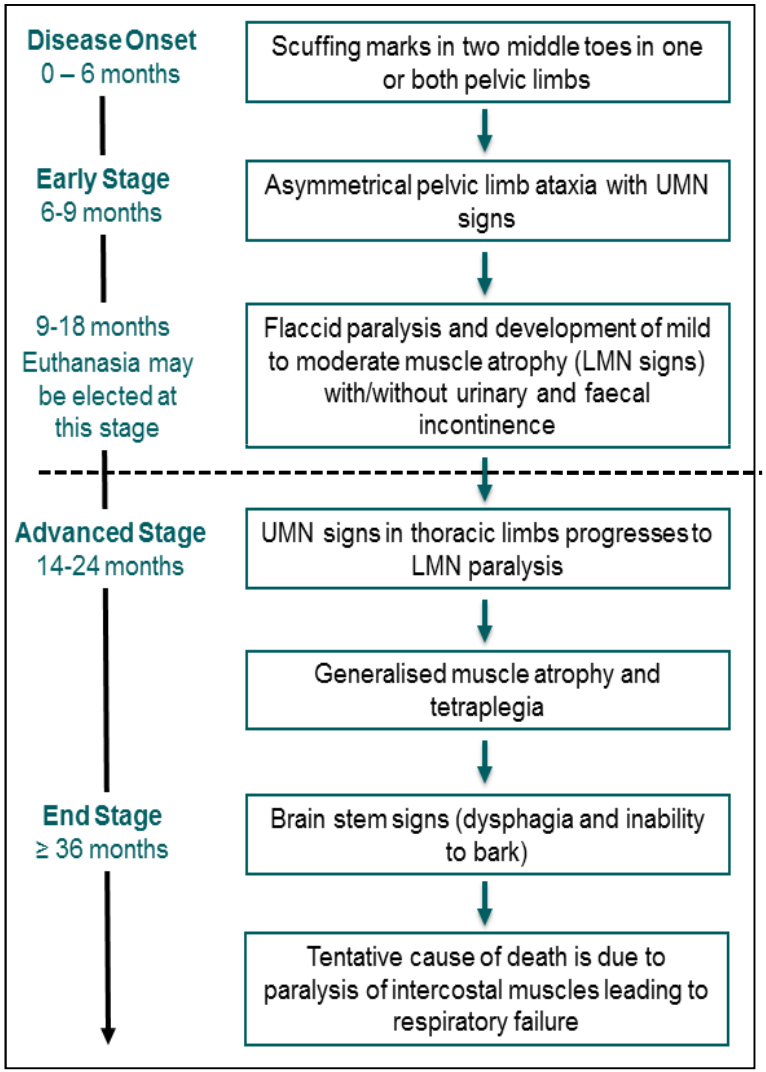
DM is a neurodegenerative conditions affecting the spinal cord of adult/aged dogs

- 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 *Sod*1 gene



Methods Employed: investigation of disease mechanism

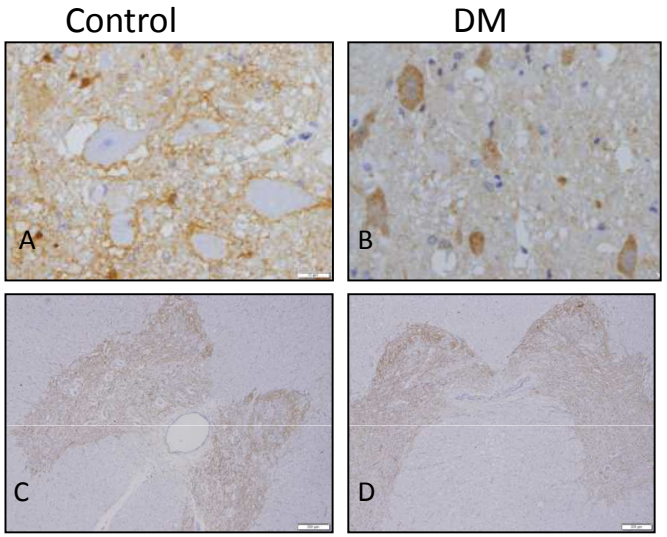
- Archive tissue bank from post mortem material
  - Pamela Johnston et al 1994-99
  - fixed and fresh frozen CNS
- Fixed material
  - Spinal cord and brain suitable for IHC studies
  - Perfusion fixed suitable for EM
- Fresh frozen material stored in LN
  - Suitable for protein expression and mRNA studies
- In vitro studies
  - Expression of tagged (GFP/Cherry) wildtype and mutant SOD1 protein
  - Neuroblastoma and motor neuron derived cells
  - SOD1 activity gels

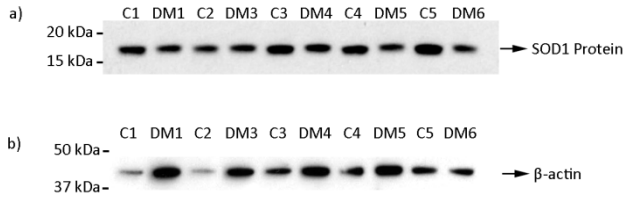
Methods employed: biomarkers identification

- 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)

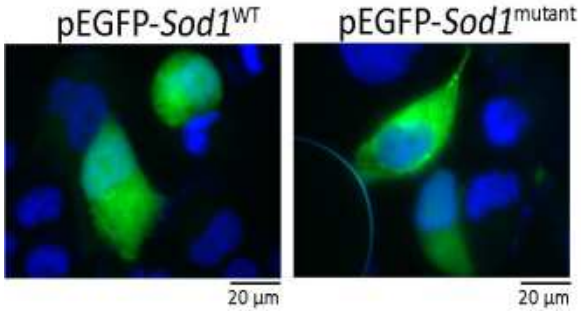
Disease mechanisms: pathology and biochemistry

- Pathology: Dr Livia Henderson (resident)
  - Assess SOD1 aggregate accumulation
  - Assess its relationship with neuronal/glia integrity
  - Non cell autonomous?
- Biochemical studies: Yao Qi (masters)





Disease mechanisms: *In vitro* studies (Yao Qi)



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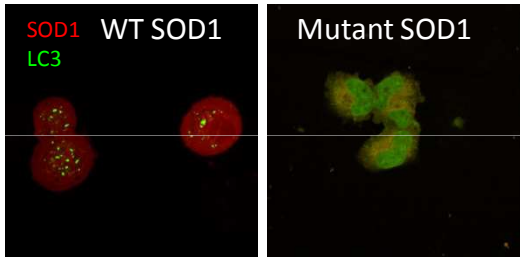

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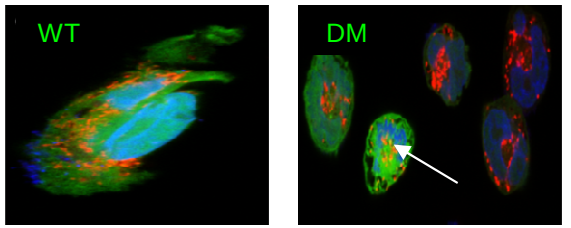
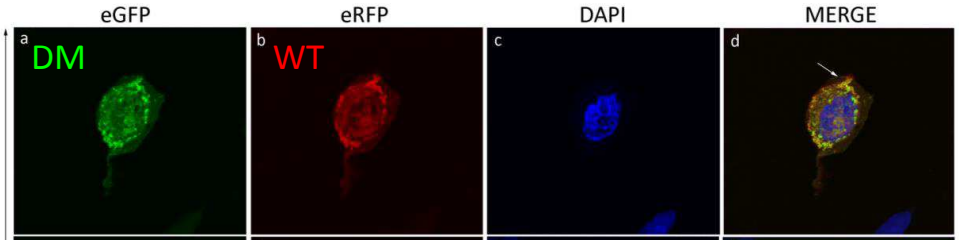
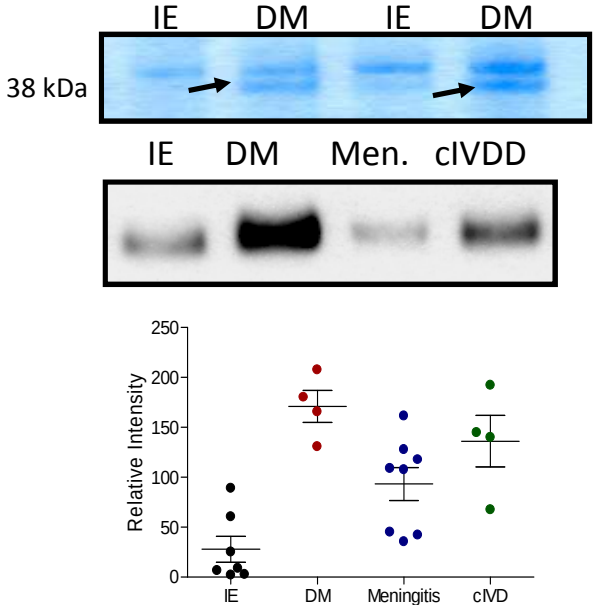


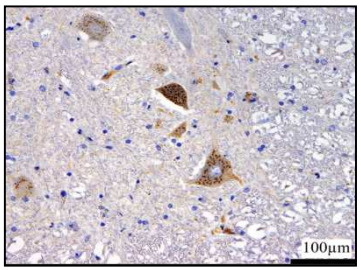


CSF Biomarker identification: is one enough?

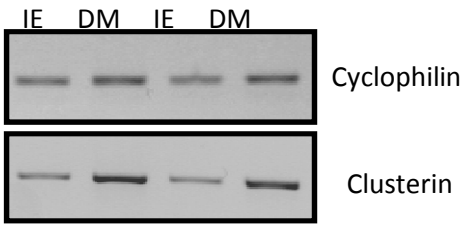
- Clusterin (apolipoprotein J)
  - A potential candidate for DM
  - But elevated in IVDD
  - Does not fulfil biomarker criteria
  - May inform on disease mechanisms
- Other candidates
  - TTR and cystatin C
- A panel of biomarkers are required



Clusterin IHC



Clusterin mRNA



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