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INVESTIGATION OF THE PATHOGENESIS OF SUSPECTED INHERITED NEUROLOGICAL DISEASES IN AUSTRALIAN SHEEP

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SUMMARY

Several cases of neurological diseases in sheep were submitted opportunistically to a government veterinary diagnostic laboratory in Australia. Initial analysis suggested a possible genetic cause for segmental axonopathy, degenerative thoracic myelopathy, lissencephaly and cerebellar hypoplasia and cervicothoracic vertebral subluxation. Suitable case material is available and will be investigated further using in depth pathological investigation to establish diagnostic criteria, understand pathogenesis and propose candidate genes. Whole genome sequencing data will be used to identify likely causal variants with the aim to develop diagnostic tools for industry.

INTRODUCTION

The Online Mendelian Inheritance in Animals database (OMIA, https://omia.org/home/) lists 194 inherited defects in sheep and likely causal variants have been identified for only 32 of these to date. A number of suspected inherited conditions have been reported recently in sheep in Australia and New Zealand. Our group has identified causal variants for neuronal ceroid lipofuscinosis (OMIA 001443-9940, Tammen *et al.* 2006), brachygnathia, cardiomegaly and renal hypoplasia syndrome (OMIA 001595-9940, Woolley *et al.* 2020) in Merino sheep and hydrops foetalis/pulmonary hypoplasia and anasarca (OMIA 000493-9940) in Persian sheep, but several suspected inherited neurological conditions are still under investigation (Woolley *et al.* 2019). Characterisation of inherited diseases and identification of causal variants is imperative to the development of diagnostic capabilities to identify affected and carrier animals and inform better management and breeding practices.

This study focussed on a detailed literature review of four diseases to inform further characterisation of the phenotype and pathogenesis to assist with selection of confirmed cases for genetic analysis, and identification of candidate genes associated with disease by comparison to similar genetic conditions in animals and humans.

MATERIALS AND METHODS

Review of previously submitted case material. Selected suspected inherited neurologic diseases for which case material was available at the Elizabeth Macarthur Agricultural Institute, Department of Primary Industries, NSW (EMAI) were further characterised based on clinical presentation, clinical pathology, gross pathology, histopathology and where indicated, special stains, immunohistochemistry, transmission electron microscopy and additional diagnostics. Case material was both retrospective from previous submissions received at EMAI, and prospectively recruited from emergent disease cases submitted to EMAI during the study period. Upon identification of suspected heritable neurological conditions, ongoing investigation involves exclusion of differential diagnoses presenting with similar clinical and/or pathologic abnormalities. Existing SNP chip and whole genome sequencing (WGS) data from previous analysis were catalogued.

Structured literature review. A structured literature review of selected neurological conditions was conducted. PubMed, Web of Science and OMIA searches were used to identify relevant

literature. Published literature was evaluated for each disease to identify new references and summarise current knowledge about disease phenotypes, mode of inheritance and candidate genes associated with disease. New information was used to update entries in OMIA. The literature was also reviewed to identify any additional suspected or known inherited neurologic phenes in sheep.

RESULTS AND DISCUSSION

Review of previously submitted case material. Suspected inherited neurological diseases identified for initial investigation included ovine segmental axonopathy and degenerative thoracic myelopathy using retrospective analysis of case material, cervicothoracic vertebral subluxation from both retrospective and current investigations, and a recent investigation of lissencephaly and cerebellar hypoplasia diagnosed in a flock of crossbred sheep in NSW (Table 1).

Table 1. Inherited ovine neurologic conditions investigated: available case material, whole genome sequencing (WGS) and SNPchip genotyping data

Disease	OMIA ID	Case material	WGS / SNP50* data
Segmental axonopathy	001492-9940	Multiple animals from several properties	1 affected sheep (MGIseq System, Q30% = 88.05)
Degenerative thoracic myelopathy	000079-9940	Multiple animals, 1 property	None
Lissencephaly and cerebellar hypoplasia	001867-9940	Multiple animals, 1 property	1 affected sheep (MGIseq System, Q30% = 88.28)
Cervicothoracic vertebral subluxation	002313-9940	Multiple animals from several properties	2 affected sheep (Illumina HiSeqTM X Ten, Q30% = 92.16%); 9 affected & 2 obligate carriers (SNP50)

*all WGS = 150bp paired-end reads, 30X coverage; SNP50 = Illumina® OvineSNP50 Genotyping BeadChip (CA, USA).

Structured literature review. A literature review did not yield any additional references for lissencephaly and cerebellar hypoplasia or cervicothoracic vertebral subluxation that were not already listed in OMIA. One review article referencing degenerative thoracic myelopathy and two review articles referencing segmental axonopathy were added to OMIA.

Ovine Segmental axonopathy ('Murrurundi disease') has been reported in Merino sheep of 1 to 5 years of age in Australia and New Zealand (Hartley and Loomis 1981; Harper *et al.* 1986). Clinically, sheep present with gradually progressive hindlimb ataxia (Hartley and Loomis 1981). Gross post-mortem lesions are absent or limited to hindlimb muscle atrophy (Harper *et al.* 1986; Jolly *et al.* 2006; Windsor 2006). Histologically, affected animals have vacuolation and spheroid formation throughout white matter in the brain and spinal cord (Hartley & Loomis 1981; Harper *et al.* 1986) with Wallerian degeneration of variable severity. Within the spinal cord, dorsal columns are more severely affected with spheroids compared to ventral and lateral columns (Harper *et al.* 1986). Axonal swellings associated with this condition ultra structurally contain membrane-bound vesicles (Jolly *et al.* 2006; Windsor 2006) and mitochondria (Windsor 2006). It has been postulated that the vesicles may originate from degenerating organelles (Jolly *et al.* 2006; Windsor 2006). Proteomic analysis has found cytoskeletal abnormalities in the trigeminal root, thought to be secondary changes (Jolly *et al.* 2006). It has been suggested that ovine segmental axonopathy may be an autosomal recessive inheritance (Jolly *et al.* 2006).

Degenerative thoracic myelopathy has been reported as a cause of hindlimb ataxia or paresis in Australian Merino sheep (Harper *et al.* 1991). Affected animals were between 5 and 24 months of age (Harper *et al.* 1991). Clinically, animals have slowly progressive hindlimb ataxia and paresis

with neurologic examination consistent with a thoracolumbar lesion. Histologically this disease manifests as symmetrical Wallerian degeneration of variable severity, predominately affecting the ventromedial and dorsolateral tracts of the spinal cord (Harper *et al.* 1991). As this is a non-specific lesion, exclusion of differential diagnoses is essential in the investigative process, with potential differential diagnoses including plant toxicities, were excluded in this study (Harper *et al.* 1991). While a hereditary cause of degenerative thoracic myelopathy is suspected, it has not yet been proven.

Lissencephaly and cerebellar hypoplasia has been identified in mixed breed sheep on a property in New South Wales in 2019. Preliminary pathological investigation resulted in a diagnosis of LCH. LCH has previously been identified in Spanish Churra lambs (Pérez *et al.* 2013; Suárez-Vega *et al.* 2013), as well as humans, goats (Santos *et al.* 2013) and calves (Santos *et al.* 2016). In Churra lambs, affected animals were markedly ataxic and died within days of birth (Pérez *et al.* 2013). There was marked cerebellar hypoplasia, agyria and pachygyria with reduced and disorganised layers within the cerebral cortex, and disorganisation of the hippocampus histologically (Pérez *et al.* 2013). A monogenic autosomal recessive pattern of inheritance was suspected, with affected animals shown to have a 31-bp deletion in predicted exon 36 of the *RELN* gene and an absence of the protein reelin, a reported cause of LCH in humans (Pérez *et al.* 2013; Suárez-Vega *et al.* 2013). LCH in humans has also been associated with variants in genes including *DCX* and *LIS1*, although additional genetic variants are suspected (Ross *et al.* 2001).

Cervicothoracic vertebral subluxation has been reported in Poll Merino sheep in NSW (Hill *et al.* 1993; Cronin *et al.* 2019), Corriedale sheep in NSW (Hartley *et al.* 1994), Columbia lambs in the US (Lakritz *et al.* 1995) and Suffolk sheep in Scotland (Nisbet and Renwick 1961). Affected animals range in age and typically show a dropped or U shaped neck with hindlimb ataxia and variable neck rigidity or pain and inspiratory stridor (Hill *et al.* 1993; Hartley *et al.* 1994; Cronin *et al.* 2019). The consistent gross finding in this condition is subluxation or deviation at the junction of the cervical and thoracic vertebrae leading to spinal cord compression (Hill *et al.* 1993; Hartley *et al.* 1994; Cronin *et al.* 2019). Some reports have described gross changes in the paravertebral muscles including white streaks and pinpoint haemorrhages, characterised histologically by muscle degeneration, necrosis, regeneration, mineralisation, haemorrhage and fibrosis (Cronin *et al.* 2019). Cronin *et al.* (2019) postulated this was an inherited condition based on pedigree analysis; however, the responsible variant(s) remains to be determined. Woolley *et al.* (2019) reported on the findings of initial investigation of SNP genotyping and whole genome sequencing data, but a likely causal variant has not been identified.

Ongoing research. The literature review identified strong candidate genes for LCH and standard analysis of WGS of a single affected animal identified possible likely causal variants that are currently validated. Current investigation involves immunohistochemistry to describe the condition and compare the reported cases to the disease in Churra lambs and humans.

For the remaining three diseases the literature review did not identify strong candidate genes and the initial analysis of limited WGS data for cervicothoracic vertebral subluxation (Woolley et al 2019) and segmental axonopathy was inconclusive. Detailed analysis of the histopathology of all identified case material will be conducted with the aim to establish clear diagnostic criteria for each disease and to accurately characterise the phenotype and underlying pathogenesis. This information will assist in diagnosis of future cases as clinical presentation alone has resulted in misdiagnosis of neurological conditions in the past. Furthermore, possible candidate genes can be identified by comparison to similar genetic conditions in animals and humans.

DNA from six additional affected animals (2 each for segmental axonopathy, degenerative thoracic myelopathy and cervicothoracic vertebral subluxation) has been submitted for whole genome sequencing. Standard methods will be used to align reads to the ovine reference genome and to call and annotate variants. This new data, existing WGS data (Table 1) and publicly available

control WGS data will be used to filter for private variants in identified candidate genes that segregate with the disease phenotype. Likely causal variants will be validated in additional samples.

CONCLUSIONS

A number of neurological conditions recognised in sheep are suspected to be hereditary. Characterising the clinical aspects and pathology of these conditions will facilitate accurate diagnosis of affected animals, provides insight into possible pathogenesis, and can assist in guiding genetic investigations to identify causative genetic variants. This study is in the early stages and we aim to raise awareness and encourage submission of additional samples to be used in the validation stage of the genetic study.

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