Identifying the genetic cause of an internationally unique, naturally occurring muscular dystrophy in Western Australian Merino sheep *

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Muscular dystrophies (MDs) are neuromuscular disorders characterised by chronic, usually progressive, skeletal muscle weakness. Individuals often lose walking ability and can suffer terminal cardiorespiratory complications. Determining the genetics of a disease helps provide diagnosis, prognosis, genetic counselling, and the basis for rational therapeutic design.

A naturally occurring sheep model of congenital progressive MD was identified in Western Australia in the 1950s and preserved as a research colony, cared for initially at Murdoch University and later at the University of Western Australia's Shenton Park facility. During this time, the inheritance was established to follow an autosomal recessive pattern. Further investigations revealed the clinical and histological features of ovine congenital progressive muscular dystrophy (OCPMD).

OCPMD is novel with respect to its pathological features and distribution: this collection of pathologies having never before been observed in a single disease. OCPMD shares features in common with the human diseases nemaline myopathy and myotonic dystrophy, but targeted investigations prior to this project have discounted the genes involved in those pathologies as being causative of the disease.

Animal models of human disease are integral to the investigations of disease pathophysiology, as well as the development and trial of potential therapies. One of the major hurdles in the treatment of MD is delivery to the target tissue, which in humans is distributed through a much larger area relative to current most popular animal models of MD, mice. The similarity between skeletal muscle mass and distribution in humans and sheep mean that an ovine model of MD would be incredibly valuable, representing a significant improvement over those currently being utilised. Another advantage of developing a sheep model is the increased amount of tissue available for biopsy and subsequent pathophysiological investigations relative to smaller mammals. This is a major benefit as the specificity of muscle degeneration in the MDs is still poorly understood and requires further study to elucidate.

Successfully characterising the causative gene(s) in OCPMD would thus enable a possible target for new therapies and may open new lines of investigation into the better understanding and treatment of MD in humans.

The International Sheep Genome Consortium (ISGC) has been working for a number of years to develop a virtual sheep genome to enable the kinds of investigations into sheep genetics that are possible in other, better developed, genomes such as the bovine or human. A contributing member of the consortium, James Kijas (CSIRO), provided several data sources to assist our investigations: an affected and a carrier individual from the OCPMD flock were whole-genome sequenced on the Illumina platform; nine affected and five carrier individuals were SNP genotyped on the '50K SNP Chip' developed by the ISGC (containing 49 034 single nucleotide polymorphisms conserved in sheep); and the latest release of the virtual sheep genome (version 3.1.) was provided for use.

This project utilised a two-pronged approach to investigate the genetics of OCPMD. First, bioinformatics analysis of SNP genotyping for the nine affected and five carrier individuals in concert with the virtual sheep genome was undertaken, enabling homozygosity mapping, genetic linkage and association mapping to be carried out. Additional SNP genotyping was carried out later in the project to increase the statistical power, resulting in 14 affected and six carrier individuals for the final analyses. Second, molecular biological approaches were undertaken to further investigate the identified prime candidate gene by cDNA and gDNA sequencing.

This ambitious, multidisciplinary honours project combining both *in silico* and molecular techniques has identified a prime candidate gene for a causative genetic variant in the OCPMD flock: a ubiquitously expressed gene not previously associated with disease, which has a muscle-specific isoform. It has additionally provided the first evidence in sheep of this isoform, previously only reported in humans, mice and rats. Thus it has laid the groundwork for further investigations into this gene and the OCPMD pathology leading to a possible pinpointing of the causative disease mutation in future work.

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