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INVESTIGATING EMERGING INHERITED DISEASES IN AUSTRALIAN LIVESTOCK: A COLLABORATIVE APPROACH

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SUMMARY

Emerging inherited diseases can cause numerous issues for producers, including productivity loss, profit loss and animal welfare problems. Under-reporting of emerging inherited diseases can result in difficulties associated with identifying and managing these diseases. The development of a research centre between the University of Sydney and Elizabeth Macarthur Agricultural Institute, NSW Department of Primary Industries is a current collaborative effort to encourage the submission of suspected inherited disease cases. Previous collaboration has resulted in the ongoing investigation of 10 inherited diseases using SNP-based homozygosity mapping and next generation sequencing to identify positional candidate genes and causal mutations. The long-term aim is to formally develop a research centre that allows independent investigation of emerging inherited diseases in livestock that builds upon current joint research.

INTRODUCTION

Emerging inherited diseases within Australian livestock can often go unreported, either because they are misdiagnosed as non-inherited diseases or are not reported due to concerns of profit loss and reputation damage. Not reporting suspected inherited disease cases can lead to a loss of valuable sample resources and a lost opportunity to characterise the phenotype(s), thus causing a delay in investigating or monitoring these diseases. Without the assurance of a robust genotyping test to identify heterozygous individuals, the management of autosomal recessive inherited diseases can become problematic, especially if detailed pedigrees are unknown for at-risk populations (Man *et al.* 2007).

The under-reporting of suspected recessive inherited diseases can contribute to the inadvertent dissemination of deleterious alleles throughout populations. If a deleterious allele can be traced to a common ancestor within a prominent sire line, all offspring are at risk of being heterozygous for the deleterious allele and only a DNA test will be able to accurately identify true heterozygous animals. Emerging inherited disease monitoring and the implementation of management programs to avoid carrier by carrier matings are important for reducing the number of affected progeny born, as well as mitigating any production and economic losses. The importance of these management programs has been shown in the case of brachygnathia, cardiomegaly and renal hypoplasia syndrome in Merino sheep (Shariflou et al. 2013), where breeding programs have reduced the number of affected progeny born (Shariflou, personal communication).

Researchers at the University of Sydney and the Elizabeth Macarthur Agricultural Institute, NSW Department of Primary Industries (EMAI) each have a longstanding history in investigating inherited diseases in Australian livestock and have recently started to collaborate on numerous research projects. So far, 10 inherited diseases are being investigated and are likely to be inherited

via a recessive mode of inheritance: congenital mandibular prognathia (CMP) in Droughtmaster cattle, pulmonary hypoplasia with anasarca (PHA) in Persian sheep, Niemann-Pick type C disease (NPC) in Angus cattle, congenital blindness (CB) in white Shorthorn cattle, cervicothoracic vertebral subluxation (CVS) in Merino sheep, a new variant of cardiomyopathy woolly haircoat syndrome (CWH) in Hereford cattle, new variants of ichthyosis fetalis (IF) in Hereford and Shorthorn cattle, suspected cases of congenital contractural arachnodactyly (CCA) in Murray Grey cattle, ovine dermatosparaxis (OD) in Merino sheep as well as the previously reported brachygnathia, cardiomegaly and renal hypoplasia syndrome (BCRHS) in Merino sheep (Shariflou et al. 2013).

A SNP-chip based homozygosity mapping approach and next generation sequencing is described with an aim to identify positional candidate genes, identify causal mutations and develop diagnostic DNA tests. The long term aim resulting from these collaborations is to develop an independent centre where producers and veterinarians can report and submit samples of suspected inherited disease cases. The centre will follow a similar approach to previous studies conducted and will benefit the Australian livestock industries through increased awareness and acceptance of reporting.

MATERIALS AND METHODS

In current collaborative research projects, SNP genotyping was performed by the Animal Genetics Laboratory (University of Queensland, Gatton, Australia) and Australian Genome Research Facility (Westmead, Australia) (Table 1). Sliding windows of 25, 50 and 100 SNPs were used to identify runs of homozygosity (ROH) for all affected animals using the bovine UMD3.1 genome assembly and the ovine Oarv1.0 genome assembly. ROH were analysed using PLINK (Purcell *et al*. 2007) and were considered to be regions of interest if these regions were shared by all of the affected animals and not with any of the carrier and control animals. These regions were scanned for positional candidate genes based on gene function.

Table 1. Number of affected and carrier DNA samples sent for SNP chip genotyping and regions of homozygosity, including species specific OMIA ID

Disease	OMIA ID[1]	Breed	Affected /Carrier	SNP chip	Region of interest
Cervicothoracic vertebral sublaxation	000077-9940	Merino	14/2	SNP50[2]	OAR10
Pulmonary hypoplasia with anasarca	000493-9940	Persian	5/5	SNP50[2]	OAR1,3,4,6,7,9,17, 25,26
Cardiomyopathy and woolly haircoat syndrome	000161-9913	Poll Hereford	2/0	SNP80[3]	BTA1,4,6,12,15,24, 25
Congenital blindness	-	Shorthorn	2/3	SNP80[3]	BTA5,14,16,22,24
Congenital contractural arachnodactyly	001511-9913	Murray Grey	5/5	SNP80[3]	BTA21
Congenital mandibular prognathia	-	Droughtmaster	9/4	SNP80[3]	BTA26
Ichthyosis fetalis	000547-9913	Hereford	1/3	SNP80[3]	multiple
Niemann-Pick disease	-	Angus	2/2	SNP80[3]	BTA3,4,16,24,29

[1]OMIA http://omia.angis.org.au, - indicates no species specific OMIA ID. [2]SNP50 = Illumina® OvineSNP50 Genotyping BeadChip (CA, USA). [3]SNP80 = GeneSeek® Genomic Profiler Bovine HD Chip 80K chip (Neogen, NE, USA).

Sanger sequencing of select candidate genes was commenced but was cost and labour intensive. Next generation sequencing (NGS) of affected animals for CMP, CVS, PHA and BCRHS using the Illumina HiSeq™ X Ten sequencing platform was performed by the Kinghorn Centre for Clinical Genomics (Garvan Institute of Medical Research, Darlinghurst, Australia) through the Ramaciotti Centre for Genomics (University of New South Wales, Sydney, Australia) with 150bp paired-end reads at 30X coverage (Table 2). This NGS data has been aligned to either the bosTau8 or oviAri3 reference genome assemblies and will be analysed for genetic variants. Samples of affected animals for IF, CWH and OD are undergoing sequencing using an in-house Illumina HiSeq® 3000 sequencing platform in Switzerland (Table 2).

Table 2. Number of affected DNA samples for next generation sequencing

Disease	Breed	Affected	Expected coverage	% of sequences with mean Q>30
Brachygnathia, cardiomegaly and renal hypoplasia syndrome	Merino	1	30X	85.84
Cardiomyopathy and woolly haircoat syndrome	Poll Hereford	2	20X	In progress
Cervicothoracic vertebral sublaxation	Merino	2	30X	92.16
Congenital mandibular prognathia	Droughtmaster	2	30X	86.58
Ichthyosis fetalis	Hereford	1	20X	In progress
Ichthyosis fetalis	Shorthorn	1	20X	In progress
Ovine dermatosparaxis	Merino	2	20X	In progress
Pulmonary hypoplasia with anasarca	Persian	2	30X	90.17

RESULTS AND DISCUSSION

Homozygosity mapping has successfully revealed and/or excluded positional candidate genes for all of the inherited diseases currently being investigated (Table 1; Shariflou *et al*. 2013; Tammen *et al*. 2016). The known mutation for CCA in Angus cattle was confirmed to be present in the Murray Grey cattle with suspected CCA. Validation of a genetic variant in a positional candidate gene for NPC is ongoing. Partial Sanger sequencing of positional candidate genes for CVS, PHA, CMP and CWH did not reveal any disease-causing mutations and affected animals were therefore re-sequenced using NGS. Previous mapping of BCRHS did not identify a clear positional candidate gene and an affected animal sample was submitted for NGS. Known candidate genes for CWH and CB were excluded and alternate candidate genes need to be investigated within the regions of interest identified (Table 1). Strong candidate genes exist for IF and OD, as these diseases have been previously characterised in different breeds (Charlier *et al.* 2008; Zhou *et al.* 2012). The affected animals tested negative for the known disease causing mutations and were re-sequenced due to suspected genetic heterogeneity.

Preliminary quality control analysis of the NGS data is positive with per base sequence quality determined by a Phred score of Q>30 ranging from 85.84% to 92.16% (Table 2) with no over-represented sequences identified. After aligning data to the bosTau8 or oviAri3 genome assemblies, allelic variations including SNPs, indels and structural variants will be identified in the regions of interest previously identified, with a focus on positional candidate genes identified by homozygosity mapping.

The results from these studies indicate that SNP genotyping and homozygosity mapping methods are highly effective in identifying positional candidate genes for a range of disorders even

if sample sizes are small and phenotypes are poorly defined. Genome wide SNP genotyping and homozygosity mapping approaches have successfully identified candidate genes and causal mutations in a range of recessive inherited diseases in cattle, including ichthyosis fetalis in Chianina cattle (Charlier *et al.* 2008). The inclusion of NGS data to identify allelic variations will allow for several runs of homozygosity identified through homozygosity mapping to be further investigated.

CENTRE CONCEPT

The methodology framework and results described in the current research projects between the University of Sydney and EMAI demonstrates the success of the working relationship between both groups. The concept of an independent research centre geared towards the molecular characterisation of emerging inherited diseases in livestock could provide a central point of contact for veterinarians, breeders, producers and breed societies. It has the potential to increase confidential reporting of suspected cases and provide research services with the aim to rapidly develop low-cost diagnostic tests based on frameworks that are already implemented at both institutions. The availability of diagnostic DNA tests will allow for informed breeding decisions to be made to avoid potentially devastating profit loss and animal welfare issues.

The centre will aim to publish validated results which will increase awareness for the role of emerging inherited diseases within Australian livestock populations. The future development of the centre will be focussed on developing a stream-lined research and diagnostic service that may involve additional research and industry groups. The key driving factor behind successfully developing an independent centre will be the collaborative relationships and shared resources between numerous research groups to encourage greater surveillance of emerging inherited disease in livestock across NSW and nation-wide.

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