Emerging inherited diseases and animal welfare: A case study of congenital mandibular prognathia in Droughtmaster cattle

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Summary

Emerging recessive inherited diseases within livestock can have detrimental impacts upon animal welfare and can cause significant economic losses. Congenital mandibular prognathia in Australian Droughtmaster cattle is an emerging inherited disease that has a minor impact upon animal welfare. This study aimed to sequence the coding regions of the previously identified positional candidate gene FOXI2 to identify disease-causing mutations. Our results were inconclusive due to failed amplification of one of the exons. This study highlights the ease in which mapping recessive inherited diseases can be achieved, although the identification of disease causing mutations may still be challenging and can therefore delay the development of diagnostic DNA tests that can support the management of emerging inherited diseases through the improvement of animal welfare.

Introduction

Since the domestication of livestock, the controlled breeding of cattle (Bos taurus) has accelerated the genetic gain of desirable production traits (Mignon-Grasteau et al. 2005; Hayes et al. 2013). The dispersal of superior genetics has been assisted by advanced reproductive techniques (ART), however in some instances the application of controlled breeding and ART can inadvertently contribute to elevated rates of inbreeding and decreased effective population sizes, especially if popular sire lines are used frequently (Charlier et al. 2008; Whitlock et al. 2008). As a consequence, the possibility for deleterious recessive alleles to disperse and be inherited in homozygous form can increase (Whitlock et al. 2008; Windsor et al. 2011).

The molecular characterisation of 117 inherited diseases showing Mendelian inheritance in cattle (OMIA 2016) has allowed for improved management of these inherited diseases. Despite the advances in molecular and quantitative genetics, we are still aware of the under-reporting of emerging inherited diseases, which poses an issue for future management (Windsor et al. 2011). An example of an emerging inherited disease is congenital mandibular prognathia (CMP) in Australian Droughtmaster cattle. CMP is a craniofacial deformity that is characterised by the inability of the mandible and maxilla to meet uniformly. The presentation of CMP is a concern due to the inability of animals to meet their optimal nutritional requirements, which also represents an economic loss to producers through reduced production efficiency. This study aimed to sequence the coding regions of the positional candidate gene FOXI2 which was selected by using a homozygosity mapping approach of nine affected and four carrier Droughtmaster cattle on an 80K SNP chip (Tsimnadis, unpublished data) to identify potential disease-causing genetic variants.

Materials and Methods

Samples
EDTA blood samples from nine affected and four carrier Droughtmaster cattle were obtained by the Elizabeth Macarthur Agricultural Institute (EMAI), Australia from regional veterinarians and/or producers. One affected Droughtmaster, one normal Droughtmaster and one unrelated normal Angus control were used in this study and DNA was extracted as part of a previous study (Tsimnadis, unpublished data).

PCR, sequencing and bioinformatics
Bovine sequence data for the candidate gene was obtained from the National Center for Biotechnology Information (NCBI) (Pruitt et al. 2014). Primers were designed using PrimerBLAST (Ye et al. 2012) and PCRs were performed using standard protocols in a final volume of 20µL. Purified PCR products were sent to the Australian Genome Research Facility (AGRF) for Sanger sequencing with the associated forward and reverse primers. Sequencing data was analysed using Sequencer® (Gene Codes Corporation, MI, USA) and allelic variants were compared to the variations database in Ensembl (Cunningham et al. 2015).

Results and Discussion

Amplification of exon one of the FOXI2 gene failed to generate a PCR product of expected size for various primer pair combinations in affected Droughtmaster and normal Droughtmaster and Angus DNA samples. Sequencing of non-specific PCR products resulted in sequences that failed to align to the FOXI2 gene. Exon two of FOXI2 was sequenced previously with no causative mutations identified (Tsimnadis, unpublished data).

The results from this case study highlight that whilst the advancement in molecular and quantitative genetics can assist in identifying disease-causing mutations, the confirmation of FOXI2 as the disease causing gene harbouring a causal mutation for CMP has so far not been possible. Potential reasons for the failed amplification of exon one of FOXI2 include possible errors in the bovine genome sequence of FOXI2 or a differing genomic sequence of exon one from the Bos taurus reference sequence due to the Droughtmaster breed consisting of both Bos taurus and...
Bos indicus breeds (Bongso et al. 1981; Bolormaa et al. 2013). Future research centred on the re-sequencing of the candidate gene in other affected and control animals is planned.

The resultant delays for the development of a direct diagnostic DNA test can impact on producers as informed breeding decisions can become increasingly difficult, especially if reliable pedigree data is unavailable (Man et al. 2007). Consequently, affected animals are likely to be born in the future. The animal welfare implications of CMP are limited compared to other inherited diseases that are observed in livestock. This case study highlights the need to identify causative mutations for recessive inherited diseases in livestock to improve animal welfare and economic outlooks through the prevention of breeding affected animals (Healy 1996).

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