

Causative Gene Discovery for Sheep Inherited Disorders

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Summary and Implications

Chondrodysplasia, inherited rickets and lower motor neuron disease are three sheep disorders in New Zealand that have been recently shown to be inherited apparently in a recessive manner. To discover the causative genes involved in the above diseases, around 50,000 genetic differences called single nucleotide polymorphisms (SNPs) throughout the genome were tested to define homozygous regions, one of which should harbor the causative mutation. Fine mapping of these regions for each disease was performed by discovering new SNPs located on candidate genes in these regions. To date, responsible mutations were successfully identified for inherited rickets and lower motor neuron disease. Our findings will benefit sheep breeding practices by providing a tool that can be used to avoid at-risk mating and may benefit science by providing animal disease models for human studies.

Introduction

Chondrodysplasia in Texel sheep is characterized by dwarfism and angular deformities of the forelimbs. Inherited rickets in Corriedale sheep is a skeletal disease characterized by decreased growth rate, thoracic lordosis and angular limb deformities. Lower motor neuron disease in Romney sheep is characterized by normal appearance at birth, but a progressive weakness and ataxia after the first week of life and finally failure to stand up without assistance. Sheep with these diseases have high mortality rates and suffer. In order to characterize the genetic bases behind these recessive disorders, genome wide associated studies, followed by fine mapping were conducted to search for causative mutations. The ultimate goal of this research is to develop genetic tests to manage carriers of these disorders and to develop animal models for human studies.

Materials and Methods

DNA samples were collected from 23 Texel sheep for chondrodysplasia, 20 Corriedale sheep for inherited rickets, and 30 Romney sheep for lower motor neuron disease. The affected and carrier sheep for each disease represented families descended from a founder animal with the genetic defect. The genome-wide association studies were conducted using the Illumina OvineSNP50 BeadChips on the 3 groups of sheep. The IBD (identity by descent) scans examine the whole genome of the genotyped cases and

carriers, and produce a list of all the strings of more than 10 consecutive homozygous markers common to all the cases. The homozygous fragments uniquely existing in affected cases but not carriers are the targeted genome segments which likely contain the mutation. Genes in the targeted homozygous regions were examined and sequenced for causal polymorphisms concordant with disease status.

Primers were designed to amplify exons and adjacent genomic sequences for candidate genes. Polymerase chain reaction-restriction enzyme digestion (PCR-RFLP) experiments were used for genotyping. Each new SNP variant was analyzed to check whether it could induce a missense or nonsense mutation and hence the disease.

Results

One specific homozygous region presumed to harbor the causative mutation was separately identified for each of the three sheep diseases. For chondrodysplasia, a 1 Mbp homozygous region covering 25 consecutive SNPs was discovered. A 10 Mbp homozygous region of 199 consecutive SNPs was identified for inherited rickets and shown to contain one nonsense mutation responsible for the disease. A 7 Mbp homozygous region of 145 consecutive SNPs was identified for lower motor neuron disease, with one missense mutation in this region found that is likely responsible for the disease.

Discussion

Genome-wide association studies combined with homozygosity mapping are powerful for mapping traits controlled by one gene. This strategy has been used successfully in both human and animal studies. In our studies, we have shown two successful cases of using high density SNP arrays for localization and subsequent identification of a mutation locus causing recessive inherited defects. The results can be rapidly applied to avoid matings between animals that both carry the defective allele. This will improve animal welfare and decrease economic losses. More importantly, due to the size and relatively low cost of maintaining sheep carrying the mutant gene, sheep with these diseases could be used as animal models for these corresponding disorders in humans.

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