IDENTIFICATION OF THE GENE CAUSING CHONDRODYSPLASIA IN DEXTER CATTLE

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INTRODUCTION

Dexter cattle are a small breed of cattle originating in Ireland which have been bred in Australia for several decades. There have been reports of mutant, aborted foetuses in this breed of cattle, described as chondrodysplastic foetuses (or “bulldog” calves). The affected foetuses display disproportionate dwarfism, a short vertebral column, marked micromelia, a relatively large head with a retruded muzzle, cleft palate, protruding tongue and a large abdominal hernia. Dexter chondrodysplasia is inherited in an incompletely dominant manner, in which the affected foetus has two copies of the defective gene. As part of an approach to controlling the disease in Australia, Dexter Cattle Australia (DCA) chose to initiate and support research to develop a DNA based diagnostic test to identify carrier animals, prevent carrier x carrier matings, hence reducing the incidence of chondrodysplastic foetuses.

MATERIAL AND METHODS

Animals. A panel of 21 animals was used for the initial mapping and mutation discovery part of this study. The relationship of the animals is shown in Figure 1, 7 affected calves, 11 carrier animals and 3 unrelated control animals. For validation of the disease causing mutation, eight affected calves from the UK, three from Germany and three from New Zealand were analysed.

Candidate gene identification and homozygosity mapping. A targeted homozygosity mapping approach was used to identify chromosomal regions with known candidate genes. Eleven candidate genes were identified based on their known involvement in bone growth and development. The estimated chromosomal position of the candidate genes on the cattle genome was accomplished with the use of bovine and human comparative maps at the Bovine Genome Database (http://bos.cvm.tamu.edu/htmls/HBM.html.) (Band *et al*., 2000; Chowdhary *et al*., 1996 ; Hayes, 1995 ; Solinas-Toldo *et al*., 1995).

The panel of 21 Dexter DNA samples was used for a homozygosity mapping approach using 68 microsatellite markers. There are three popular AI bulls in Australia which are all proven carriers, AI1, AI2 and AI3. Figure 1 shows the initial pedigree available for analysis including the 3 carrier bulls. Both AI1 and AI2 are 5th generation descendants of the same bull. However, AI3 does not appear to be related to this founder bull and is a crossbred bull from a Dexter x Jersey mating.

Linkage mapping. To map the selected candidate gene to the cattle linkage map, the International Bovine Reference Panel (IBRP) was used (Barendse *et al*., 1997). A non-coding single nucleotide polymorphism (SNP) was found in the sequence of the Angus control animal and this polymorphism was used to map the gene to the cattle linkage map.

Mutation analysis. For mutation detection in the candidate gene identified through homozygosity mapping, two affected calves were used, along with a proven carrier animal, and a control animal (Angus breed). The entire coding region of the gene was sequenced for mutations.

RESULTS AND DISCUSSION

Eleven candidate genes were identified as most likely involvement in chondrodysplasia and their location on the bovine map was predicted. Homozygosity mapping was performed in regions predicted to contain candidate genes. The technique of homozygosity mapping relies on the fact that the DNA surrounding the disease gene will be identical by descent (IBD) (Lander and Botstein, 1987). Therefore, markers near the disease gene would show homozygosity in affected animals, but not in parents or controls. A potential homozygous region amongst the affected animals was identified. Affected animals B1 – B5 (descendants from sires AI1 and AI2) were homozygous for a single allele for the three markers of interest, whilst carrier and control animals were heterozygous. Samples B6 and B7 (descendants from bull AI3) were not homozygous, a total of 3 different alleles in affected animals for each of these three markers. The LOD score for two of the markers was 2.11, which is positive, although not significant due to small family size. As B6 and B7 are unrelated to the family used, this region was still identified as a region of interest.

The location of the candidate gene predicted to map to the region of interest was confirmed by linkage mapping. Sequencing of the candidate gene revealed a 4 bp, GGCA insertion in the affected samples. To genotype samples for this mutation, fragment size analysis was conducted using polyacrylamide gel analysis on an automated sequencer (LI-COR), as shown in Figure 2. The 4 bp insertion mutation causes a frame shift which lead to a premature termination codon. The resulting protein is predicted to contain less than one third of the original amino acid sequence.

In summary, of 24 affected calves, 21 calves that are not related to the bull AI3 or his female line are tested to have two copies of the mutation, genotype bb. This includes affected samples from New Zealand, Germany and the UK. All 22 parents of the homozygous affected calves are heterozygous for the mutation, genotype Bb. Of the 293 other Dexters for which DNA samples were collected during the study, 218 are homozygous normal (BB), and 75 are heterozygous carriers (Bb).

Bull AI3 and both his parents are all homozygous normal for this mutation. Two affected calves, sired by bull AI3 have a genotype of Bb and two affected calves with AI3 on both sides of their pedigree have a genotype of BB. The minimum prevalence of heterozygotes carrying the second mutation is estimated at approximately 1.7% in Australia assuming it was only introduced by AI3. Due to herd book restrictions in other countries, it is likely that the second mutation is only found in Dexters in New Zealand and Australia as AI3 was a crossbred bull originating from a Jersey Red Poll x Dexter.

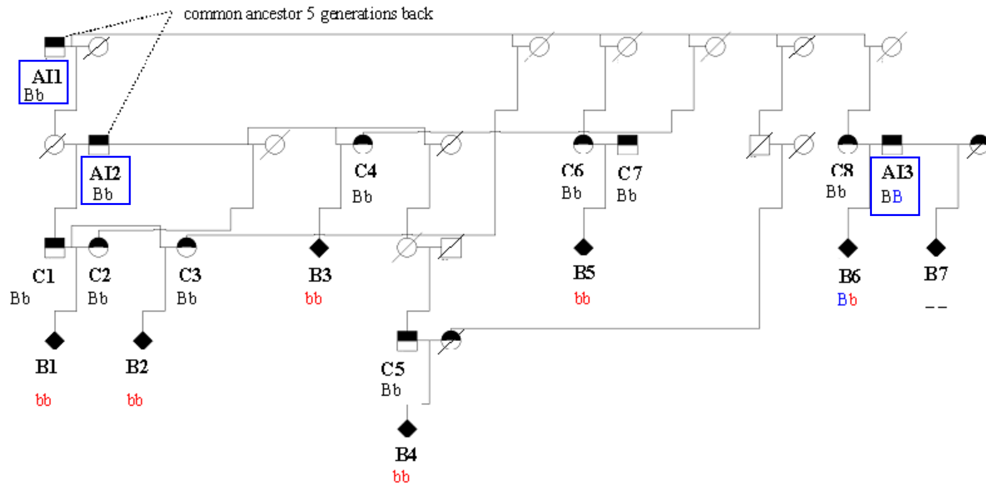

Figure 1. Pedigree of Australian Dexter families including 7 affected calves (B1-B7). The pedigree was drawn using PEDRAW (Curtis, 1990). The letters below the animal ID indicate alleles for the mutation. Note that AI3 and B6 do not conform. Key : ○ = female, □ = male, ◆ = unknown sex, half-filled = carrier, filled = affected, line through symbol = sample not available

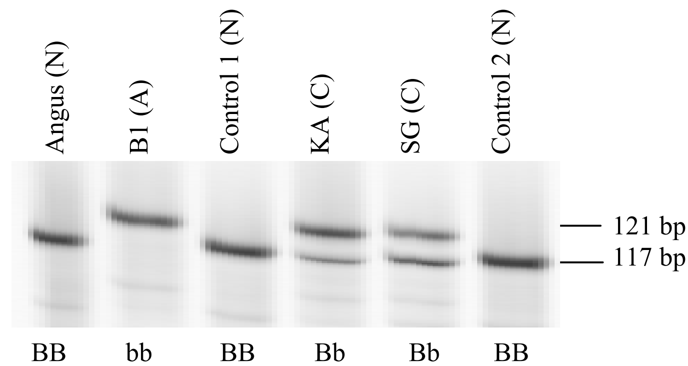

Figure 2. Chondrodysplasia DNA test - fragment length analysis of 4 bp insertion mutation. The identification of each animal is shown at the top of the image (with phenotype in brackets, N : normal, A : affected, C : carrier). The genotype is shown at the bottom of the image, and the fragment sizes on the right-hand side.

CONCLUSION

Based on a candidate gene /homozygosity mapping approach we have successfully identified a putative causative gene for Dexter chondrodysplasia A 4 bp deletion in a homozygous form is associated with severe chondrodysplasia in calves and is consistent with the introduction of a premature stop codon leading to a truncated gene product. The mutation in a heterozygous form is associated with a varying degree of dwarfism as shown by physical measurements (data not shown) although, not all heterozygotes show visible or measurable dwarfism. This is consistent with the incomplete dominant mode of inheritance. A second mutation causing chondrodysplasia in Dexter cattle, as yet unidentified, is thought to only occur in Australia and New Zealand. This mutation is likely to affect the same candidate gene and has been introgressed from a genetic source outside the Dexter breed.

Dexters have been selected for their shorter, often dwarf-like appearance usually indicative of a carrier animal. The DNA-test will enable informed mating decisions, avoidance of carrier x carrier matings and reduced incidence of chondrodysplasia-affected calves. A major benefit of the test will be the ability to select desirable breeding animals of a short stature that do not carry the chondrodysplasia defect.

Finally, there is potential for Dexter chondrodysplasia to be used as a model for human dwarfism. Screening of the gene in patients with homologous forms of dwarfism may reveal a disease causing mutation.

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