HOMOZYGOSITY MAPPING APPROACH FOR THE CHONDRODYSPLASIA GENE IN DEXTER CATTLE

J. A. L Cavanagh[1], I. Tammen[1], P. A. W. Harper[2], H. Raadsma[1], F. W. Nicholas[3]

[1]Department of Veterinary Clinical Sciences, University of Sydney, Camden, NSW, 2570
[2]NSW Agriculture, Grafton Agricultural Research&Advisory Station, Grafton, NSW, 2460
[3]Department of Animal Science, University of Sydney, NSW, 2006

SUMMARY

Dexter cattle are a dwarf breed of cattle originating in Ireland and 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 (also known as "bulldog" calves), displaying disproportionate dwarfism. Dexter chondrodysplasia is considered to be inherited in an incompletely dominant manner. The homozygous affected foetus is aborted in mid to late gestation with occasional affected calves born alive and dying shortly after birth. As part of an approach to controlling the disorder in Australia, the Australian Dexter Association (ADA) has chosen to support research aimed at development of a DNA test to prevent carrier/carrier matings.

As DNA was only available from a few affected foetuses, a homozygosity mapping approach in combination with candidate gene regions was adopted to localise the disease gene. A total of 90 microsatellite markers were selected to cover specific regions. A selection of animals was genotyped and the results analysed by searching for regions of homozygosity in the affected samples. Of the 90 selected markers, one demonstrated a homozygous pattern amongst the affected samples, but not among the parents and unrelated animals. Genes in this homozygous region need to be identified in cattle. Mutation testing of gene(s) in the homozygous region can then begin to bring us closer to a DNA-based heterozygote test and the cause of chondrodysplasia in Dexter Cattle.
Keywords: Dexter, cattle, chondrodysplasia, genetics

INTRODUCTION

Dexter cattle are a popular breed in Australia, but are often discriminated against as they are known to be carrying a lethal genetic defect, chondrodysplasia. It would appear that two A.I. sires carrying the defect have been excessively used in Australia with a number of "bulldog" calves produced, and an estimated minimum heterozygote frequency calculated at 19% (Harper *et al.* 1998). There exists a widely accepted but not scientifically proven hypothesis that the carrier animals are generally "short-legged", as opposed to the "long-legged" normals. Therefore, most Dexter breeders adopt the advice promoted by the ADA and avoid mating two short-legged animals together to reduce the prevalence of "bulldogs". This appears to have reduced the number of "bulldogs" below the number expected.

Symes (pers.comm.) conducted an experiment in which physical size measurements of animals were recorded to predict the carrier status of the animal. Nicholas *et al* (1996) analysed the measurement data and concluded that it has potential to identify non-carriers, but is not an accurate test. A DNA-

test would be accurate, and easy for breeders, as hair samples can be used for the test, but it requires the disease-causing mutation to be identified.

A mutation in fibroblast growth factor receptor 3 (FGFR3) has been identified as the cause of achondroplasia in humans (Shiang *et al.* 1994). Therefore, Usha *et al* (1997) sequenced the transmembrane region of FGFR3 in Dexter cattle for mutations. The sequence was identical for both normal animals and "bulldog" calves, indicating that mutations in this region of FGFR3 were not responsible for Chondrodysplasia in Dexter cattle. A homozygosity mapping approach to the problem (Houwen *et al.* 1994; Charlier *et al.* 1996), combined with candidate gene selection is an efficient method to find the gene responsible for chondrodysplasia.

MATERIALS AND METHODS

Sample Collection. Samples were collected from "bulldogs", parents of "bulldogs", other relatives of "bulldogs", and distantly related animals (controls). The relationship between 7 of the "bulldogs" is shown in Figure 1. DNA was extracted from tissue samples (both formalin fixed and fresh) blood, semen and hair samples.

Post-mortems were performed on "bulldog" foetuses to confirm the diagnosis. All "bulldog" foetuses had parentage verification performed, allowing us to assume the parents of "bulldogs" as carriers of chondrodysplasia.

We are currently assembling a database containing various size measurements of many Australian Dexters and their DNA samples. The measurements taken were height at hip, length of animal, girth, cannon bone length and cannon bone circumference. The measurements were taken by the same 2 researchers independently for all animals. These data will be continually added to and analysed after the mutation responsible for chondrodysplasia in Dexters is identified.

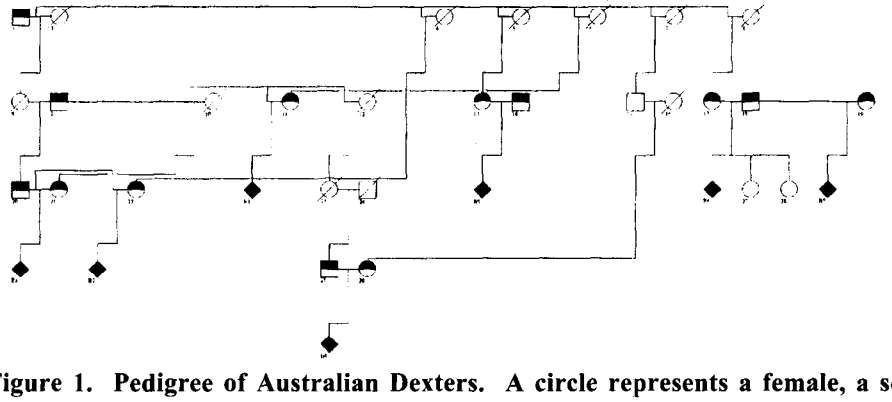

Figure 1. Pedigree of Australian Dexters. A circle represents a female, a square a male, a filled-in diamond a "bulldog", and a half-filled-in symbol a carrier. A symbol with a line through it indicates unavailability of sample. Several animals have common ancestors that have been omitted to reduce the complexity of the pedigree.

DNA analysis. 12 candidate genes were identified by searching for diseases in humans and mice in which a similar phenotype to Dexter "bulldogs" was described. It is possible that one of these genes may cause chondrodysplasia in Dexters. Using comparative mapping, nine regions on the cattle genome homologous to the candidate genes were identified. A total of 90 microsatellite markers were selected to cover these regions. DNA extraction from 22 animals was performed using QIAamp Tissue Kit (QIAGEN). The 22 animals included 11 parents or grandparents, 8 "bulldog" samples, and 3 distantly related animals to act as controls. Genotyping using a standard protocol was performed on both a Licor semi-automated sequencer and an ABI sequencer. The genotype data was analysed for regions of homozygosity amongst the affected samples. Assuming an incomplete dominant mode of inheritance, we would expect DNA markers adjacent to the disease causing mutation to show homozygosity in affected animals, whereas carriers or unaffected animals will show heterozygosity.

RESULTS AND DISCUSSION

Clinopathological Findings. Considerable variation in foetal size was observed due to different stages of abortion, varying in crown-rump length from approximately 14cm for a mummified foetus to as large as 23cm for a "bulldog" delivered at term. However, the foetuses all shared the characteristic features of considerable smallness for gestational age, short vertebral column, short limbs, a relatively large head with retruded muzzle, cleft palate and protruding tongue, a large ventral abdominal hernia and sparse hair growth for early abortions. The ventral halves of the lobes of the lungs were irregularly divided, to give their margins a festooned appearance. Individual bones within the small limbs of both foetuses had a maximum length of 15 mm, and diameters of 5-10 mm. The bones lacked distinct epiphyseal growth plates. The retarded size and histological features of these bones are considered consistent with a failure of endochondral ossification following primary ossification of the diaphysis of the cartilage model (Harper *et al.* 1998).

Genotyping Data. There have been 70 markers genotyped to date. By visual examination, only one of these markers (marker E) shows a homozygous pattern amongst 5 (B1-B5) of the 8 "bulldogs", and yet a variety of alleles amongst the parents and controls (Table 1). For this marker, "bulldog" B6 has one allele in common with the other "bulldogs" B1-B5, but the allele from the father (bull no. 18) is different. "Bulldog" B7 also has the same allele as "bulldog" B6 (from the same father) and a different allele from its mother. It is not known where bull no. 18 and cow no. 19 inherited the "bulldog" gene from, whereas bulls no. 1 and 2 have a common ancestor who was a carrier. Results for "bulldog" B8 could not be obtained as it is a degraded formalin-fixed sample. CRI-MAP (http://www.caos.kun.nl/tutorials/genomics/CRI-MAP.doc.html) was used to estimate recombination frequencies between markers and the chondrodysplasia locus: marker E gave a positive LOD score of 2.11, as did an adjacent marker F (3.3cM from E), which was analysed after the initial screen. Although the LOD score of 2.11 is not significant, these preliminary results justify further investigation of this region. To confirm these initial results, more samples from relatives are currently being collected and genotyped.

Table 1. Number of alleles per group of animals for a selection of 6 markers (A-F) Markers E and F are located on the same chromosome, markers A to D are found on other bovine chromosomes.

Group and No. of alleles per group	Markers					
	A	B	C	D	E	F
Dexter "bulldogs" (n=12)	**4**	**6**	**6**	**5**	**2**	**2**
Dexter Parents (n=22)	5	7	7	8	7	6
Dexter Controls (n=6)	4	3	3	4	3	2
Total alleles (n=40)	6	7	8	8	7	6

Future Applications. If further genotyping of additional animals confirms that the chondrodysplasia gene is linked to markers E and F, candidate genes in the area can be screened for disease causing mutations. Heterozygote testing will allow identification of the chondrodysplastic genotype of Dexter animals and an accurate prevalence of the disorder determined in Australian Dexters.

Dexter breeders all over the world will benefit from an accurate DNA test, as they will be able to prevent carrier/carrier matings, thus eliminating the occurrence of "bulldogs".

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