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Short Communication

Coat-colour dilution and hypotrichosis in Hereford crossbred calves

RD Jolly[*§], JL Wills[†], JE Kenny[‡], JI Cahill[‡] and L Howe[*]

Abstract

AIM: To investigate cases of coat-colour dilution and hypotrichosis in a group of Hereford x Friesian crossbred calves, and to define the underlying molecular genetics of the disorder.

METHODS: The investigation was predicated on the hypothesis that this disorder was similar to a known dominantly inherited disorder of calves of black breeds crossed with Simmental cattle, for which there were candidate gene mutations. Sequence analyses of PCR amplicons from exon 1 and exon 11 of the premelanosome protein 17 gene (*PMel17*) were carried out. Restriction enzyme digestions of amplicons were followed using electrophoresis of digested fragments.

RESULTS: It was shown that an affected calf and its Hereford sire were heterozygous for a three-base deletion in exon 1 of the *PMel17* gene. These two animals were also heterozygous for a second mutation in exon 11 of the *PMel17* gene. Four other related animals were likewise heterozygous for both mutations in the sire's herd of origin.

CONCLUSIONS: Coat-colour dilution and hypotrichosis in Hereford crossbred calves in New Zealand is the same genetic disorder as that previously described in Simmental crossbred calves, and is linked to mutations in the *PMel17* gene.

KEY WORDS: *Hypotrichosis, coat-colour dilution, Hereford cattle, Simmental cattle, PMel17 gene*

Introduction

Coat colour is a distinctive aspect of many breeds of cattle that helps set them apart from others. Likewise, favoured crosses between breeds may result in distinctive and recognisable colours and patterns. The genetics behind these are relatively well understood, and result from variations in genes associated with the production, packaging and export of eumelanin (black/brown) or pheomelanin (yellow/red). All animals have a base colour of either black or red, but this may be diluted by various secondary diluter genes. Patterns of white against a base colour background reflect 'non-colour' and result from an absence of melanocytes in the underlying skin. The various colours and coat-colour patterns, if accompanied by normal hair, are regarded as normal, with the exception of forms of albinism.

Hypotrichosis, a disorder in which there is a congenital deficiency of hair, has long been known as an inherited abnormality in a number of breeds of cattle including Herefords, where it is inherited as an autosomal recessive trait (Jayasekara et al 1979). Remaining sparse hair tends to be misshapen and brittle, and results in newborn calves that may suffer from cold stress, leading to death or failure to thrive under normal conditions of husbandry. This recessive disorder contrasts with a different form of hypotrichosis combined with coat-colour dilution, that occurs when red animals, particularly of the Simmental breed and carrying the mutation responsible, are crossed with black, or black pied cattle. Inherited as a dominant trait, 50% of progeny of such matings have black diluted to a charcoal or a chocolate-coloured coat, and variable degrees of hypotrichosis (Ayres et al 1989; Schalles and Cundiff 1999). This particularly affects the tail-switch, which may be deficient in hair, resulting in a 'rat-tail' appearance that has given the disorder the colloquial name of 'rat-tail syndrome'. However, any white areas of the coat, including tail-switch, have normal hair. Light and scanning electron microscopy show the shape of the affected hair to be short, curled, occasionally crimped, asymmetrical in diameter and taper, and sometimes twisted. Transmission electron microscopy shows the melanin granules are enlarged, aggregated and irregularly distributed in the hair shaft relative to those of normal hair (Ayres et al 1989).

In a genetic study of an experimental Simmental crossbred herd, the coat-colour dilution/hypotrichosis phenotype segregated with a three-base (CTT) deletion at nucleotide 54 in exon 1 of the *PMel17* gene (Hecht 2006). Although the study was not conclusive, this is the presumptive mutation for the disorder. A second single nucleotide polymorphism, a C→A transition in exon 11 in the second nucleotide of codon 612 of the same *PMel17* gene, segregated with most, but not all, animals with the same phenotype. The effect of this latter mutation is uncertain.

This present report concerns an investigation of a similar syndrome of coat-colour dilution with hypotrichosis in calves born as a result of mating a Hereford bull with Friesian cows, in the Bay of Plenty, New Zealand, which resembled that seen in Simmental crossbred calves.

Materials and methods

Animals and tissues

Approximately 20 calves from 60 Friesian cows mated to two Hereford bulls were born with coat-colour dilution and hypotrichosis. Ear-punch samples from the two possible sires and affected calves were submitted to a commercial laboratory for par-

[*] Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Private Bag 11222, Palmerston North, New Zealand.
[†] PO Box 333, Matamata, New Zealand.
[‡] Equine Parentage and Animal Genetic Services Centre, Massey University, Private Bag 11222, Palmerston North, New Zealand.
[§] Author for correspondence. Email: r.d.jolly@massey.ac.nz

bp	Base pair(s)
PMel17	Premelanosome protein 17
RFLP	Restriction fragment length polymorphism

entage testing. At a later date, similar samples were taken from one remaining affected calf and the bull identified as his sire, for further investigation of the defect. Tissue from a Friesian calf from another property was used as a control. Blood samples in EDTA vacutainers from the sire's herd of origin were also submitted for investigation.

Amplification of DNA sequences using PCR

Ear-punch samples were processed for DNA using the DNeasy Tissue Kit (Qiagen, Victoria, Australia), as per the manufacturer's instructions. Water blanks were included as sample processing controls, to confirm the lack of contamination during the sample manipulation process.

PCR assays to amplify the exons 1 and 11 were performed as described previously (Hecht 2006), with minor modifications. Primer sequences are listed in Table 1. The 30-µl PCR reaction mixture contained 2 µl of test sample DNA (50 ng/µl total DNA), 1 x PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.2 µM forward and reverse primers, and one unit of platinum Taq DNA polymerase (Invitrogen, Carlsbad CA, USA). The thermal cycle conditions were as follows: 95°C for 5 min; 35 cycles at 94°C for 1 min, then 56°C for 30 sec and 72°C for 1 min; and one cycle at 72°C for 10 min. Products of PCR were run on a 1.5% (w/v) ultra-pure agarose gel (Invitrogen) containing ethidium bromide, and visualised under ultraviolet light on a transilluminator.

Positive PCR amplicon samples were purified (PureLink PCR purification kit; Invitrogen), and subjected to automatic dye-terminator cycle sequencing using BigDye (Terminator v3.1 Ready Reaction Cycle Sequencing kit) and the ABI3730 genetic analyser (Applied Biosystems Inc, Foster City CA, USA), to confirm genomic sequence.

Restriction enzyme digestion of amplified DNA sequences

Approximately 100 ng of each of the exon 1 amplicons were digested with six units of MboII enzyme (New England Biolabs, Ipswich MA, USA) at 37°C for 2 h. The exon 11 amplicons were digested with eight units of BlpI (New England Biolabs) at 37°C overnight. Digested fragments were run on a 1.5% (w/v) ultra-pure agarose gel (Invitrogen) containing ethidium bromide, and visualised under ultraviolet light on a transilluminator.

Results

Clinical aspects

Several calves with coat-colour dilution were killed at birth as they were small, weak and severely hypotrichotic. Affected calves that survived were examined at 4–10 weeks of age, and noted to have short sparse curly coats of a red/brown to chocolate colour; in contrast, white hair, including that of the tail-switch, was of normal length and texture (Figure 1). The breeder considered that the affected calves did not thrive as well as normal calves in the group, but on weaning they were able to be sold for finishing. The sire of the affected calves was identified using commercial DNA-based parentage verification technology.

Sequence analysis of exon 1 of the *PMel17* gene

Sequence analysis of the amplified 293 base pairs (bp) of exon 1 DNA of the *PMel17* gene had high homology with the known *Bos taurus* sequence (GenBank Accession No. EF363684). The amplified exon 1 sequences from the affected calf and sire revealed a three-base deletion (CTT) at nucleotide 54 in one copy of the gene when compared with that of the control calf and the reported sequence. This deletion results in the loss of a recognition site for the restriction enzyme MboII and, by removal of codon 18 from the gene, the amino acid leucine is thus missing from the expressed premelanosome protein 17 (Table 2).

Table 1. Primer design table with primer sequences and approximate product sizes for PCR amplification of DNA sequences of exons 1 and 11, encoding for coat-colour dilution and hypotrichosis in cattle.

Primer pair	Primer sequence	Product size
Exon 1		
Forward	5'- GAG GGG AGG AAG GGC TAT G -3'	293 bp
Reverse	5'- AAT CAA ATG GGT GGG AGA CA -3'	
Exon 11		
Forward	5'- GAG CCA GGA TCA AGA CCA AG -3'	425 bp
Reverse	5'- CCT CCA CCC CTT AAG TGA CA -3'	

bp = base pairs

Figure 1. Hereford crossbred calf with coat-colour dilution and hypotrichosis. White areas of hair are normal.

Sequence analysis of exon 11 of the *PMel17* gene

Sequence analysis of the amplified 425 bp of exon 11 DNA of the *PMel17* gene had high homology with the known *Bos taurus* sequence (GenBank Accession No. EF363684). Sequence analysis of the crossbred calf and his sire revealed a single missence mutation in one copy of this exon, resulting in a C→A substitution in codon 612 (Table 2). This single nucleotide polymorphism would result in deletion of a recognition site for the restriction enzyme BlpI, and the substitution of glutamic acid for alanine in the expressed premelanosome protein 17. The control Friesian calf did not have the mutation.

Herd-of-origin investigation

The pedigree of the bull that had sired affected calves was known, and it was possible to investigate the status of animals related to him in his herd of origin. His sire was dead, but PCR/restriction enzyme fragment length polymorphism (RFLP) testing of 11 female progeny showed all to have a normal *PMel17* exon 1 genotype, so he was presumed normal. The dam was alive, and shown to carry one copy of the exon 1 deletion mutation. Her sire was progeny-tested, and of a further 15 daughters, one (095) was shown to carry a single copy of the exon 1 mutation. This sire was also considered normal as the dam of 095 had another hetero-

Table 2. Sequence analysis of exon 1 and exon 11 of the premelanosome protein 17 (*PMel17*) gene[a].

Exon	Amino acid sequences																	
1	^{16}Glycine			Valine			Leucine			Leucine			Alanine			Valine^{21}		
Normal sequence	G	G	T	G	T	T	C	T	T	C	T	G	G	C	T	G	T	A
Mutated sequence	G	G	T	G	T	T	–	–	–	C	T	G	G	C	T	G	T	A
11	^{610}Glycine			Serine			Alanine			Valine			Proline			Leucine^{615}		
Normal sequence	G	G	C	T	C	A	G	C	A	G	T	C	C	C	C	C	T	T
Mutated sequence	G	G	C	T	C	A	G	**A**	A	G	T	C	C	C	C	C	T	T
							Glutamic acid											

[a] Amino acid sequences are listed for exon 1 and 11, and codon positions referenced from the start codon of the *PMel17* gene. Nucleic acid deletions are marked with a dash (–). The sequence variation in exon 11 is in bold and underlined. Grey shading indicates the enzyme recognition sites in exons 1 and 11 for MboII and BlpI, respectively.

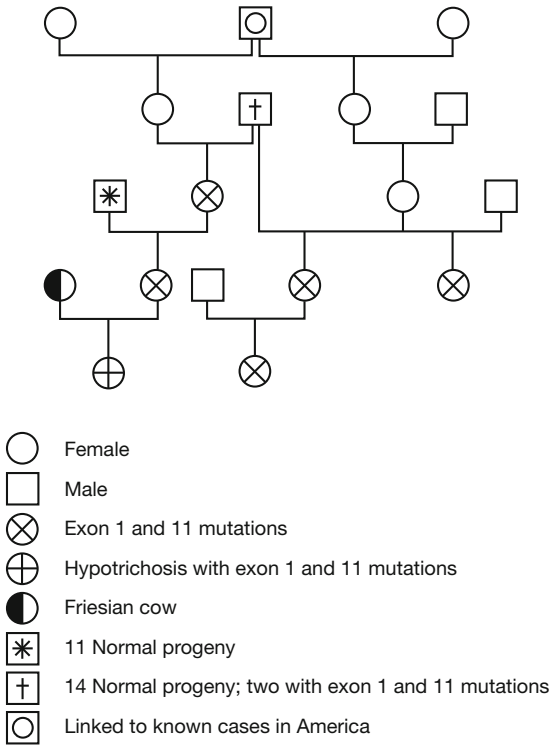

Figure 2. Pedigree of cattle related to a Hereford crossbred calf with coat-colour dilution and hypotrichosis, showing individuals that had one copy each of the exon 1 and exon 11 mutations of the premelanosome protein 17 gene.

zygous daughter by a different bull; 095 also had a carrier daughter herself (Figure 2). The mutation in the latter two animals was confirmed using direct DNA sequencing. All animals that tested as heterozygous for the exon 1 mutation were also heterozygous for the exon 11 mutation, as shown by PCR/RFLP analysis. In contrast, of 18 other animals tested in the same herd, none was associated with either mutation. The common ancestor of the cattle with the mutations was an American bull (Figure 2), the son of a bull identified to us as a carrier of this defect.

Discussion

This clinical investigation was predicated on the hypothesis that the genotype of the coat-colour dilution/hypotrichosis defect in Hereford crossbred calves was the same as that carried by Simmental cattle (Hecht 2006). The same three-base deletion (CTT) in exon 1 of the *PMel17* gene described in Simmentals was present in the Hereford bull and affected calf in the heterozygous state, and in four other related animals. The second mutation in exon 11 was likewise present in the same animals, but not in 18 other animals tested in the same herd. This confirmed that the disorder in both breeds was the same genetic entity and likewise inherited as a dominant trait in animals with a black base colour. However, the 'rat-tail' aspect of the syndrome is unlikely to be found in Hereford x Friesian crosses as both breeds have mostly white tail-switches, and white hair is normal. It would be expected in Hereford x Angus crosses, although expressivity may vary considerably, and this aspect of phenotype may not always be present (Ayres et al 1989). The phenotype of animals homozygous for these mutations has not been described, but would theoretically be more severe.

The *PMel17*-expressed protein is an eumelanosome fibrillar matrix protein on which polymerisation of eumelanin occurs (King et al 2001; Theos et al 2005). Mutations in this gene tend to cause coat-colour dilution, often to a 'silver' colour in a variety of species, and one such mutation is a major diluter gene in Charolais cattle (Kühn and Weikard 2007). All cattle are either basically black (EE, Ee) or red (ee), this being controlled by the extension locus (E) which codes for the melanocortin receptor MC1R, a G-protein-coupled receptor of melanocytes. Black is dominant to red, but may be affected by diluter genes. In black animals, melanin-stimulating hormone (melanocortin) reacts with the receptor, and through the second messenger cyclic adenosine monophosphate induces activity in the tyrosinase family of enzymes, leading to production of eumelanin (black/brown). In red cattle that have the recessive 'ee' allele coding for an inactive form of the MC1R receptor, pheomelanin (yellow/red) is produced in pheomelanosomes which essentially lack the premelanosome protein 17 protein fibrils. This explains why the syndrome is manifested in calves that are genetically black and why white hair without melanin is normal. The effect on cattle such as those of the Jersey breed, that have the wild-type E^+E^+ variant of the black gene, is uncertain.

The finding of the putative defect-related mutation in Hereford cattle allowed the development of a PCR/RFLP test that distinguished clinically normal red animals that carried the colour dilution/hypotrichosis defect from those that did not. Pedigree

analysis and PCR/RFLP, or direct DNA sequence testing in the sire's herd of origin, indicated that the defect was passed to him through a female line that had a common ancestor in an American sire, himself the son of an alleged carrier. As there are a number of descendants of these sires in New Zealand, it is likely the syndrome will occur more widely than in the present instance, particularly as Hereford bulls have a major role in cross-breeding with Friesian and Angus cattle.

The clinical syndrome of coat-colour dilution and hypotrichosis has not been reported in Simmental crossbred calves in New Zealand, but it is known to occur (RD Jolly, unpubl. obs.). Affected Simmental crossbred calves with this disorder should not be confused with similarly coloured crossbred calves with normal hair. These latter are expected to be the result of another common diluter gene that is acceptable in the Simmental breed, that dilutes red to a more yellow hue and black to a brown/chocolate colour.

The effect of this crossbred form of hypotrichosis on production is presently uncertain, but reports on affected Simmental-black crossbred calves indicated a predisposition to cold stress and poor growth rate, at least in the first year of life (Ayres et al 1989; Schalles and Cundiff 1999). In the affected Hereford crossbred calves discussed above, some were killed at birth because of small size and severe hypotrichosis. However, it was not possible to ascertain retrospectively whether other factors were involved leading to premature births, which could have confused the apparent expression of the disorder.

The exon 1 PCR/RFLP test used in this investigation was not robust, and in two cases unclear results were confirmed using direct sequencing of amplified DNA. Although tests based on DNA may be conducted on a blood sample, they are now frequently undertaken in cattle on hair bulbs from tail-switch hair. However, contamination by liquid faeces under pasture husbandry in New Zealand makes this less suitable as a source of DNA than it is in other countries, where the diet tends to be drier.

The underlying molecular information, on which this study was based, has not yet been fully validated in peer-reviewed literature, nor is it clear if the exon 1 mutation is the actual causative mutation of this syndrome. However, the problem is being examined in a separate in-depth investigation in American Hereford crossbred cattle where the same two mutations have been identified associated with the defect (JE Beever[1], pers. comm.).

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[1] JE Beever, University of Illinois at Urbana-Champaign, Urbana IL, USA