Allelic heterogeneity of Crooked Tail Syndrome: result of balancing selection?

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Summary

We report the identification of a second loss-of-function mutation (c.1906T>C) in the bovine MRC2 gene causing the Crooked Tail Syndrome in Belgian Blue Cattle. We demonstrate that the ensuing substitution of the highly conserved Cysteine 636 with Arginine causes illegitimate receptor oligomerization, which is predicted to impair function of the MRC2 encoded protein, Endo180. We propose that this second MRC2 mutation was selected by breeders as a result of its favourable effect on muscularity in heterozygotes.

Keywords

Belgian Blue Cattle, Crooked Tail Syndrome, CTS, Endo180, MRC2

We recently identified a frame-shift mutation (c.2904-2905delAG) in the MRC2 gene causing the Crooked Tail Syndrome (CTS) in Belgian Blue Cattle (Fasquelle et al. 2009). Unexpectedly, and despite the severity of the condition, we demonstrated that ~25% of Belgian Blue animals are carriers of the c.2904-2905delAG mutation. We demonstrated that this is very likely owing to the fact that the muscularity of c.2904-2905delAG carriers is enhanced, which is a highly desirable trait in Belgian Blue Cattle. Concomitantly, we demonstrated that the c.2904-2905delAG MRC2 mutation underwent a selective sweep in this breed. Since 2008, 12 000 Belgian Blue animals have been screened for the c.2904-2905delAG mutation and, by selecting against carrier progenitors and/or avoiding at-risk matings, the CTS condition has been largely eliminated from the breed.

Nevertheless, 18 new CTS cases were referred to us between November 2009 and March 2010. All of them had been sired by one of two bulls that had been tested free of the c.2904-2905delAG mutation. The symptomatology was identical to that of previously studied CTS cases, i.e. growth retardation, increased muscular development, tail deviation, stocky head, short and straight limbs, scoliosis and spastic paresis. The two sires were re-genotyped using DNA extracted from both blood and sperm and were confirmed to be free of c.2904-2905delAG. All 18 calves as well as 12 available dams were shown to be heterozygous for the c.2904-2905delAG mutation (Appendix S1).

We sequenced all 30 MRC2 exons and intron–exon boundaries in two trios and identified a novel T to C substitution in exon 13 (Fig. 1a,b). The corresponding c.1906T>C mutation is predicted to cause a disruptive substitution (p.Cys636Arg) of one of four extremely conserved Cysteines in the third C-type lectin-like domain (CTLD3), mediating intra-molecular disulphide bonds essential to stabilize the tridimensional structure of the MRC2-encoded Endo180 protein (Fig. 1d and Fig. S1) (Zelensky & Gready 2003). We developed a 5’ exonuclease assay to genotype the MRC2 c.1906T>C variant and showed (i) that both sires carried it, (ii) that all their affected offspring were compound c.2904-2905delAG – c.1906T>C heterozygotes, (iii) that the mutation was absent in a breed diversity panel comprising 141 animals representing 11 breeds other than Belgian Blue and (iv) that it had a frequency of ~0.3% (20 carriers in 3188 normal individuals) in Belgian Blue (Appendix S1). RT-PCR amplification of MRC2 transcripts from skeletal muscle of a compound heterozygous case revealed near-complete absence of c.2904-2905delAG-bearing transcripts, hence confirming their degradation by non-sense mediated RNA decay and demonstrating higher stability of c.1906T>C-bearing transcripts (Fig. 1c).
Next we studied the impact of the new mutation on Endo180 protein stability and subcellular localization. We transfected NIH-3T3 mouse fibroblasts with a pcDNA3 vector expressing full-length human Endo180 protein with either the wild-type (WT) Cysteine or the mutant (MUT) Arginine at the corresponding amino acid position [introduced by site-directed mutagenesis (c.1903-1905TGT>CGC)]. Western blot analysis under reducing conditions showed a single expected 180 kDa band expressed at similar levels in WT and MUT cell lysates but not in mock transfected lysates, indicating that the Arginine mutation does not destabilize the Endo180 protein. Interestingly,
under non-reducing conditions, in addition to the 180 kDa band, two high molecular bands were clearly visible for the MUT form but absent in the WT (Fig. 2a and Appendix S1). These likely correspond to dimers and possibly oligomers of MUT receptors, formed by inter-molecular disulphide bonds between unpaired CTLD3 Cysteines. Oligomerization was confirmed with a second Endo180 antibody (Fig. 2b).

Immunofluorescent staining of transfected cells showed the expected distribution of WT Endo180 protein to discrete intracellular endosomes present throughout the cytoplasm but concentrated perinuclearly (Sheikh et al. 2000; Howard & Isacke 2002). By contrast, the MUT protein showed a more diffuse localization, pointing to a defect in receptor trafficking (Fig. 2c).

Taken together, our data suggest that oligomerization of the Endo180 636R allele compromises its functionality, explaining the MUT phenotype of compound c.2904-2905delAG – c.1906T>C calves.

The frequent bursts of genetic defects in livestock are due primarily to the excessive use of popular sires by artificial insemination. As a consequence, defects are nearly always allelically homogeneous, and it is this feature, typical of genetic isolates, that renders them easy to map in

these populations (Charlier et al. 2008). However, if loss-of-function mutations in specific genes confer a selective advantage to carriers, breeders will pickup independent mutations even if initially very rare in the population. It is this phenomenon that underlies the occurrence of an allelic series of loss-of-function mutations in the MSTN gene in both cattle and sheep (Georges 2011). We previously showed that functional MRC2 hemizygosity increases muscle mass. We suspect that the second c.1906T>C MRC2 mutation was most likely picked up by perceptive breeders as a result of this pleiotropic effect.

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**References**


**Supporting information**

Additional supporting information may be found in the online version of this article.

*Figure S1* Position specific scoring matrix (PSSM) analysis of the mutant C636R CTLD3 domain.

*Appendix S1* Supplemental material and methods.

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