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Background

Bovine achondrogenesis type II is also known as bulldog calf (BD) and is caused by a congenital chondrodysplasia characterized by disproportionate growth of bones, resulting in a shortened and compressed body, mostly because of the reduced length of the spine and the long bones of the limbs.[1] Moreover, severe facial dimorphisms, e.g. palathoschisis and shortening of the viscerocranium, are present.[1] Recessively inherited variants in the *ACAN* gene are associated with the so-called lethal Dexter BD type[2] (OMIA 001271-9913), whereas dominant inherited *COL2A1* variants are related to forms of bovine achondrogenesis type II or BD (OMIA 001926-9913). The latter may be inherited from mosaic parents[3-6] or due post-zygotic *de novo* muations.[5, 7, 8] So far, a total of seven pathogenic variants in the *COL2A1* have been reported.[3-8]

Own analysis

A stillborn purebred Holstein male calf was delivered after dystocia. Gross pathology findings revealed a phenotype resembling the bovine chondrodysplasia type II with the additional presence of perosomus elumbis (Fig. S1). WGS was performed using genomic DNA obtained from the ear tissue of the calf as described before.[7] The sequenced reads were mapped to the ARS-UCD1.2 cattle reference genome,[9] resulting in an average read depth of approximately 19.8×. The WGS data of the case can be found in the European Nucleotide Archive under the sample accession no. SAMEA7690227. A deleterious variant in the *COL2A1* gene was hypothesized to be causal. Therefore, integrative genomics viewer software[10] was used for visual inspection of variants in the region of the *COL2A1* gene on chromosome 5. A single pair of reads indicated the presence of a 6.7 kb-sized deletion, and the sequence coverage within the deleted segment is apparently depleted compared with the flanking regions (Fig. S1). The deletion spanning 19 coding exons of the *COL2A1* gene was subsequently evaluated by performing a multiplex PCR across both breakpoints, revealing an additional PCR product only in the affected calf (Fig. S1). The two obtained PCR products across the breakpoints represent the wt allele, and thereby we confirmed that the case showing a third PCR product was indeed heterozygous for the suspected structural variant. Sanger sequencing revealed the precise breakpoints of the heterozygous deletion from position 32 301 911 located in intron 25 to 32 308 589 located within exon 45. The 6679 bp deletion includes the entire sequence of 18 exons (26–44) plus the first 36 nucleotides of exon 45 (Fig. S1).

Comments

The heterozygous deletion is predicted to affect a large portion of the *COL2A1* gene. Two possible scenarios could cause the observed phenotype: either haploinsufficiency or co-expression of a significantly truncated protein. The *COL2A1* gene has a probability of loss-of-function intolerance score of 1, meaning that it clearly falls into the class of loss-of-function haploinsufficient genes.[11] Consequently, the observed phenotype could be explained by the non-expression of the mutant allele. This has been speculated recently as a cause for a BD case showing a heterozygous 3.5 kb deletion

encompassing 10 exons of *COL2A1*.[8] On the other hand, the variant detected herein is deleting 1413 bp of the coding sequence representing an in-frame deletion that affects 438 residues of the triple helical region of the COL2A1 protein. Owing to the lack of suitable material, it remains unclear whether this shortened transcript encoding a significantly truncated protein is expressed or not. Nonetheless, the invariant Gly-x-y structural motif is mandatory for perfect triple-helix formation and could thus lead to extensive overmodification. It could be speculated that the variant allele disrupts the triple-helical region of alpha 1 (II) chain causing a dominant-negative effect similar to most of the alterations responsible for achondrogenesis/hypochondrogenesis type II in human patients (OMIM 200610).

Interestingly, so far phenotypically indistinguishable cases of BD calf syndrome in Holstein cattle have shown notable allelic heterogeneity. This report presents another large structural variant affecting the *COL2A1* gene causing a novel form of bovine achondrogenesis type II that occurred in combination with the perosomus elumbis (PE) phenotype (OMIA 000789-9913). Bovine PE has been known as a congenital entity for a long time and shows a certain morphological variation among cases in Holstein cattle, but so far no molecular cause has been reported.[12] Based on our findings, we postulate the COL2A1 gene as a possible candidate gene for PE in cattle.

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Supporting Information

Figure S1. *COL2A1* deletion in a Holstein calf with achondrogenesis type II and persomus elumbis.

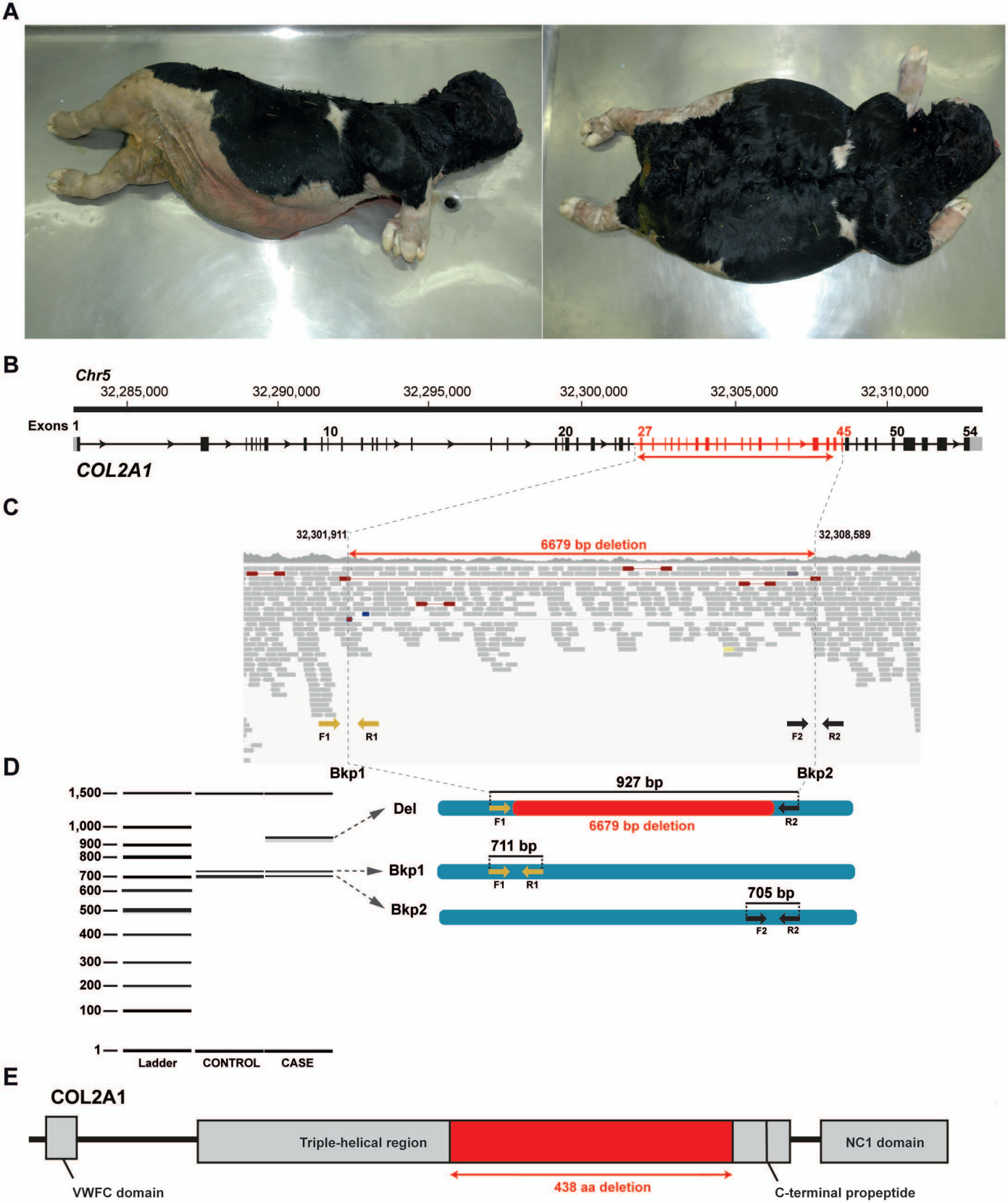

Figure S1. *COL2A1* deletion in a Holstein calf with achondrogenesis type II and perosomus elumbis. (A) Phenotype of the affected Holstein calf. (B) *COL2A1* gene structure showing the deletion on chromosome 5 (red arrow). (C) Integrative Genomics Viewer (IGV) screenshot showing the breakpoint regions represented by a decrease on the coverage and the identified 6679 bp heterozygous deletion between the two breakpoints. (D) PCR-based genotyping of the identified deletion. (E) Domain and region information for the α1 chain of type II collagen obtained from the UniProt database (http://www.uniprot.org/; accession number: P02459).