A de novo missense mutation of COL1A1 causes Osteogenesis Imperfecta type 2 and premature delivery in Normande Cattle

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C. Grohs[1*], J. Corbeau[2], J. Jourdain[3], M. Boussaha[1], V. Plassard[4], A. Barbat[1], C. Hamelin[5], J. Mortier[4], D. Boichard[1], R. Guatteo[2] and A. Capitan[1,3]

[1] Université Paris Saclay, INRAE, AgroParisTech, GABI, 78350, Jouy-en-Josas, France ; [2] BioEpAR, INRAE, Oniris, CS, 40706 Nantes, France ; [3] ALLICE, 75012 Paris, France ; [4] Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, France ; [5] INNOVAL, 35538 Noyal sur Vilaine, France ; *cecile.grohs@inrae.fr

Abstract

Seventeen male and female calves showing shortened gestation length and multiple fractures at birth were reported to the French National Observatory of Bovine Abnormalities (ONAB) among the descendants of a single Normande sire, suggesting autosomal inheritance with mosaicism. Clinical examination revealed clinical signs consistent with type II osteogenesis imperfecta (OI). Detailed analysis of gestation length and perinatal mortality supported a moderate proportion of affected calves (7.7%; 107 animals) and highlighted the underreporting of this congenital defect to the dedicated observatory. Genetic mapping, whole genome sequencing, and subsequent genotyping of cases and control animals identified a *de novo* missense substitution within the NC1 domain of *COL1A1* (Chr19 g.36473965G>A; p.D1412N). The same mutation has been reported several times to cause type II OI in human. In conclusion, we report the fourth mutation of *COL1A1* associated with type II OI in cattle and an interesting model for a syndrome also observed in human.

Introduction

In vertebrates, osteogenesis imperfecta (OI) constitutes a group of at least 15 genetic disorders characterized by bone deformity and fragility with various degrees of severity and possible addition of other symptoms (Sillence *et al.*, 1979; Besio *et al.*, 2019). Studies in humans have demonstrated that only two genes coding for the alpha 1 and alpha 2 chains of collagen type I account for 77% of cases, the rest being caused by mutations of at least 17 other genes (e.g. Marini *et al.*, 2007; Bardai *et al.* 2016). To date only three independent cases of OI have been characterized at the molecular level in cattle, all caused by *COL1A1* mutations (Bourneuf *et al.*, 2017; Jacinto *et al.*, 2021; Petersen *et al.*, 2019). From 2017 to 2019, 17 purebred Normande calves (9 males and 8 females) with symptoms consistent with OI were reported to the French National Observatory for Bovine Abnormalities (ONAB; doi.org/10.15454/BRKOV3). All cases were direct descendants from the same healthy Normande AI bull (called "Ly.") suggesting an autosomal dominant inheritance with somatic or germline mosaicism. The purpose of this study was to characterize the clinical and genetic features of this new syndrome.

Materials & Methods

Phenotypes. One affected calf and an unrelated control that died of natural death at one week of age were frozen at -20°C and subjected to various analyses (CT scan, X-ray, necropsy, reassembly of limb bones and histology of bones and tendons). Gross clinical description and DNA samples were also available for 16 additional affected half-sibs. In addition, information on the date of birth, survival within 48h after delivery, and gestation length were recovered for 1,387 progeny of Ly. and 161,413 Normande calves from 63 control bulls born the same year as Ly. (mean = 2,439 +/- 1,747 progeny per sire).

Genetic analyses. The 17 cases, 84 half-sib controls and Ly. were genotyped with various SNP arrays (Illumina, San Diego, USA) and then phased and imputed to the Bovine SNP50 in the framework of the French genomic evaluation as described in Mesbah-Uddin *et al.* (2019). Then, paternal phases were screened for sliding haplotypes of 50 markers that were carried by all cases and by less than 50% of their half-sib controls. We subsequently sequenced the genome of one case using the Illumina NovaSeq platform (150 bp paired-end reads), processed the data in accordance with the guidelines of the 1,000 Bull Genomes Project (Hayes and Daetwyler), and used 5,116 genomes from run 9 of the latter project as controls. A candidate mutation in the *COL1A1* gene was genotyped by PCR amplification with primers GCCTCCCAGAACATCACCTA and CTTTTCGGGGGTTTCAGTTT using Go Taq Flexi DNA polymerase (Promega), followed by Sanger sequencing (Eurofins MWG, Ebersberg, Germany).

Results

At necropsy, the frozen specimen presented light blue sclerae and dentinogenesis imperfecta with grey and friable teeth (not shown). CT scan and preparation of the skeleton revealed the existence of ancient fractures associated with bone loss or abnormal repair mainly located on the diaphysis of long bones, as well as recent limb and rib fractures probably due to the mechanical constraints of calving (Figure 1a-d).

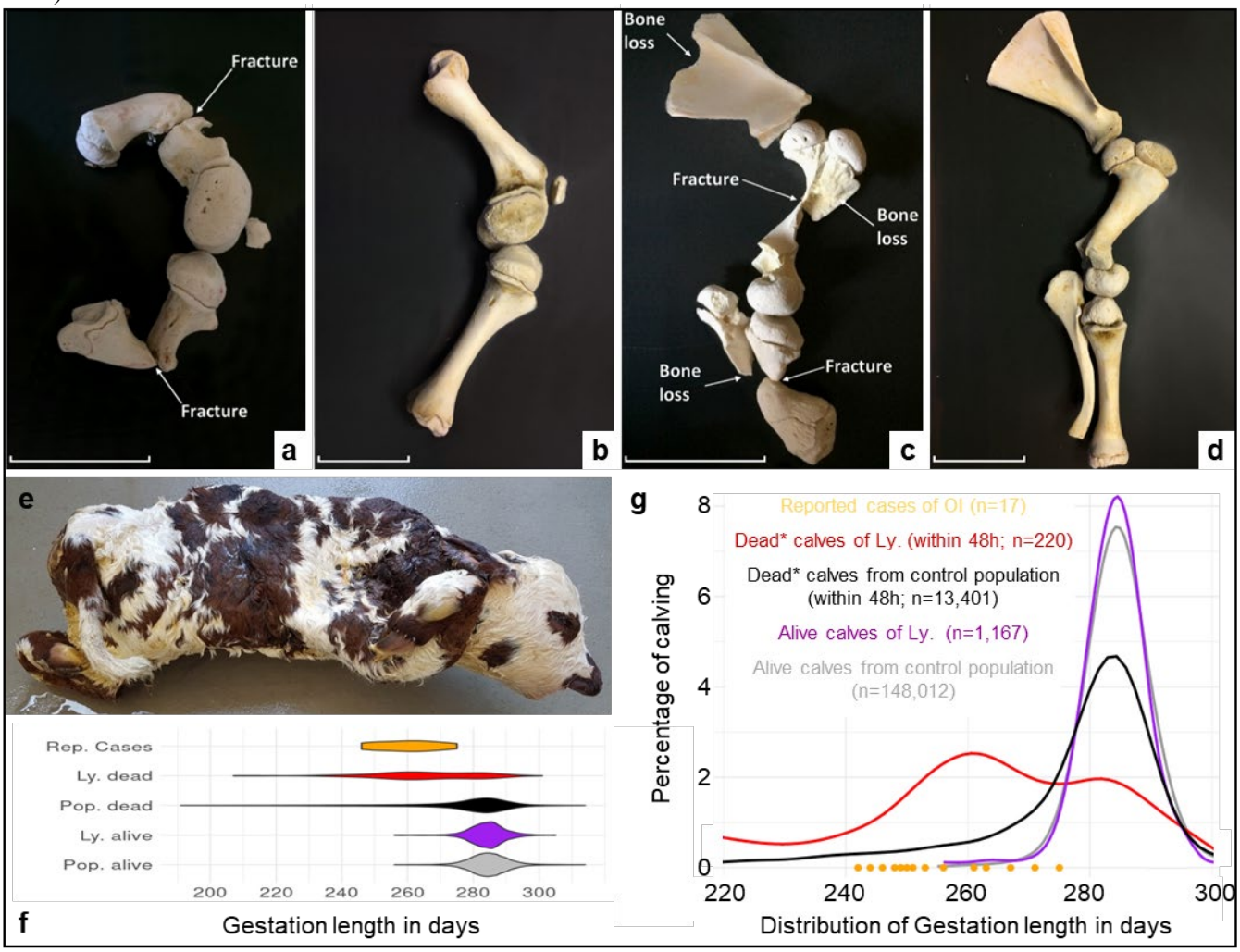

Figure 1: Clinical manifestation of OI. Details of bones from posterior (a,b) and anterior (c,d) limbs of case (a,c) and control (b,d) calves; general view of an OI calf (e); statistics on gestation length and perinatal mortality within the descendants of Ly. and control bulls (f) and related distributions (g).

This calf also suffered from arthrogryposis associated with a thickening of the joint capsules, and from a generalized osteopenia as revealed by a comparative x-ray analysis with a control calf (not shown). These symptoms fitted perfectly with the clinical features of the most severe form of OI, namely type II. The 17 cases all died at birth or soon after, and displayed twisted or broken limbs, as well as reduced gestation length (Figure 1e-g). Ly. was found to be the worst Normande bull for perinatal mortality with 15.9 % of calves declared as dead within 48h after birth versus 8.2 $\pm$1.5 % for 63 control sires. Assuming that this excess of perinatal mortality was due to OI, we estimated the proportion of affected calves to be 7.7 % (*i.e.* 15.9-8.2 %) among its progeny. This would represent 107 cases, which contrasts with the only 17 reported to us and highlights the underreporting of congenital defects to heredosurveillance platforms, even for textbook genetic syndromes. The gestation lengths of the perinatally dead calves of Ly. were significantly shorter than those of matched control calves ($p < 2.2\text{^x } 10^{-16}$; T test ; Figure 1f) and showed a bimodal distribution with an additional subpopulation centered around 260 days that comprised the reported cases of OI (Figure 1g). This observation is in agreement with previous reports of premature rupture of membranes in sporadic cases of OI due to collagen defects, leading to preterm delivery (e.g. Cole *et al.*, 1992).
To gain insights into the genetic architecture of OI in this family, we analysed phased Bovine SNP50 genotypes from the 17 cases and 84 half-sib controls. Assuming autosomal dominant inheritance, we mapped the locus to chromosome 19 between positions 35,459,825 and 41,972,985 bp (ARS-UCD1 assembly). Then, by comparing whole-genome sequences of one case and 5116 controls representing the worldwide cattle diversity, we identified a single heterozygous candidate variant in this interval: a missense variant affecting an aspartic acid residue within the NC1 domain of COL1A1 that is entirely conserved among vertebrates (Chr19 g.36473965G>A ; p.D1412N ; SIFT score = 0 ; Figure 2).

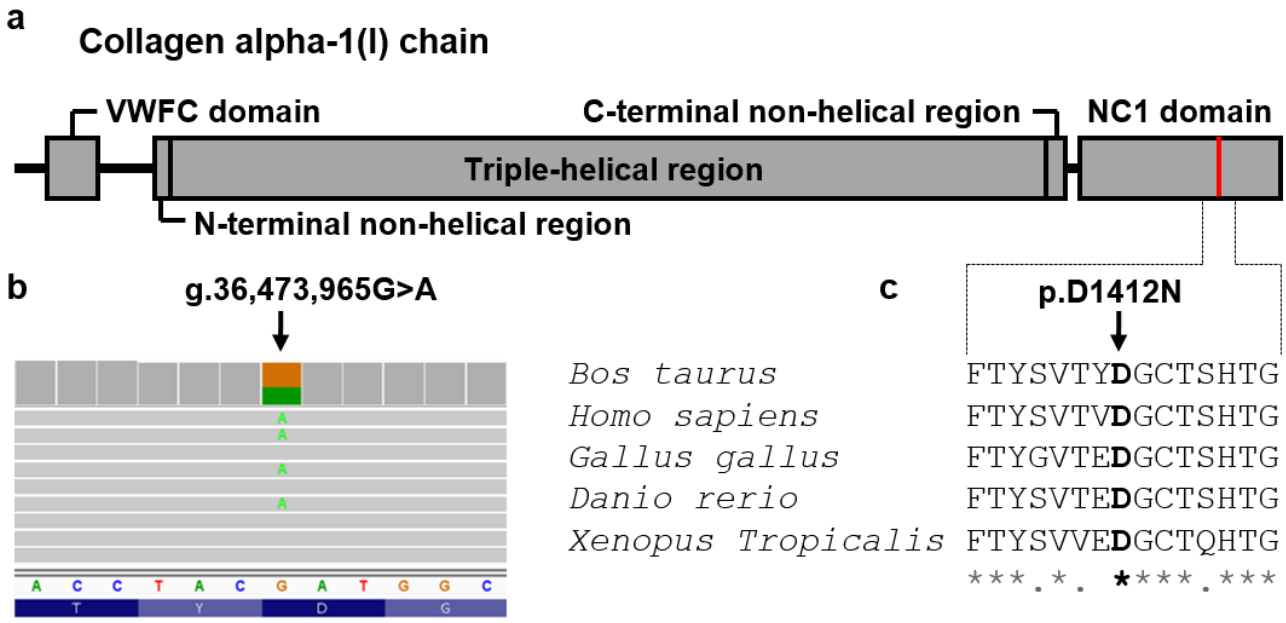

Figure 2. Details on the candidate variant. Functional domain of the bovine COL1A1 protein (a), Integrative genomic viewer screenshot showing the substitution at the DNA level (b), and multiple alignment of COL1A1 ortholog proteins from various vertebrate species (c).

Interestingly, the same substitution has been reported three times (e.g Bodian *et al.*, 2009) to be responsible for type II OI in human which supports its causality. As a further verification we genotyped the sire, 3 affected calves and 3 controls carrying the same paternal haplotype (but in the non mutated version) using PCR and Sanger sequencing and confirmed the *de novo* nature of this mutation. Finally, based on the small deviation of the 1:1 ratio for each paternal haplotype among the

84 control animals (i.e. 40 vs 44) we estimated a proportion of affected calf of 4.5 % (4/88) which is consistent with our previous estimation of 7.7 % based on the excess of perinatal mortality.

Conclusion

In conclusion, we identified a *de novo* deleterious substitution of COL1A1 that (i) is responsible for type II OI associated with preterm delivery in the progeny of a mosaic bull and (ii) corresponds to a mutation previously described in human patients suffering from the same syndrome. This study highlights the interest of large data sets available in livestock species to characterize genetic defects and in parallel raises the concern of underreporting of genetic defects to dedicated observatories.

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