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Report of a bovine form of Ehlers-Danlos Syndrome in Red Holstein cattle due to a *de novo COL5A1* nonsense variant

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Introduction

Ehlers-Danlos syndrome (EDS) encompasses a group of painful, hereditary connective tissue disorders, characterised by skin hyperextensibility, atrophic scarring and generalised joint hypermobility. In humans, an international classification of fourteen subtypes has been established (Malfait *et al.,* 2017; Malfait *et al.,* 2020), based on clinical presentation and varying degrees of severity. To date, more than twenty associated genes have been identified which encode fibrillar collagens, as well as various collagen-interacting proteins. The Online Mendelian Inheritance in Animals (OMIA; Nicholas *et al*., 1995) database lists similar syndromes in eight animal species, including cattle (Colige *et al*., 1999; Carty *et al*., 2016; Jacinto *et al*., 2020). In livestock, such defects often result in increased juvenile mortality due to infection or euthanasia, making the identification and management of these conditions a critical issue for breeding companies. In 2021, the French National Observatory for Bovine Abnormalities (ONAB) was asked to investigate cases of a condition resembling EDS observed in the offspring of a Red Holstein sire (called "N.") used for artificial insemination (AI). Our aim was to comprehensively characterise the EDS-like phenotype and elucidate its genetic basis.

Materials & Methods

Animals. A cohort of 16 affected and 113 control descendants of "N." was assembled for genetic investigations by combining spontaneous reports, a breeder survey of progeny genotyped for routine genomic evaluation, and survival data. Three affected animals were hospitalised and necropsied at Alfort National Veterinary School (ENVA, France).

Histological analysis. Healthy and lesioned skin were collected from the head, neck, back, and ear tip for two of the individuals. In addition, control skin samples were collected on two unaffected Holstein cows at ENVA. Samples were fixed in 10% formalin, dehydrated through an ethanol series, cleared in xylene, embedded in paraffin, and sectioned at 5 µm. Sections were mounted on adhesive slides and stained with haematoxylin-eosin-safran.

Genetic analysis. Assuming dominant inheritance, transmission disequilibrium testing (TDT) was conducted on phased Bovine SNP50 array genotypes from 16 affected and 113 healthy direct descendants of the sire, following preprocessing with the French genomic evaluation pipeline (see details in Mesbah-Uddin *et al.* 2019). Paternal allele transmission

was compared between groups using Fisher's exact test with Bonferroni correction. Whole-genome sequencing of one affected animal (13.6x coverage) was performed on an Illumina NovaSeq6000 at the GeT-PlaGe facility (http://genomique.genotoul.fr). Heterozygous Single Nucleotide Polymorphisms (SNPs) and small Insertion-Deletions as well as structural variants located in the 95% confidence interval (CI) of TDT mapping were assessed against 1,367 control genomes from our local database (Boussaha *et al*., 2016), comprising 348 Holstein individuals and representatives of 25 additional breed. A strong candidate variant was genotyped by Polymerase Chain reaction (PCR) followed by Sanger sequencing on 3 case and 3 unaffected half-siblings carrying the at-risk haplotype. Finally, the degree of germline mosaicism in the sire was estimated from allele transmission at two markers flanking the best candidate variant using the formula (nHb − nHa) / (2 × nHb), where nHa and nHb denote carriers of the at-risk and alternative paternal haplotypes among half-sibling controls, respectively.

Results & Discussion

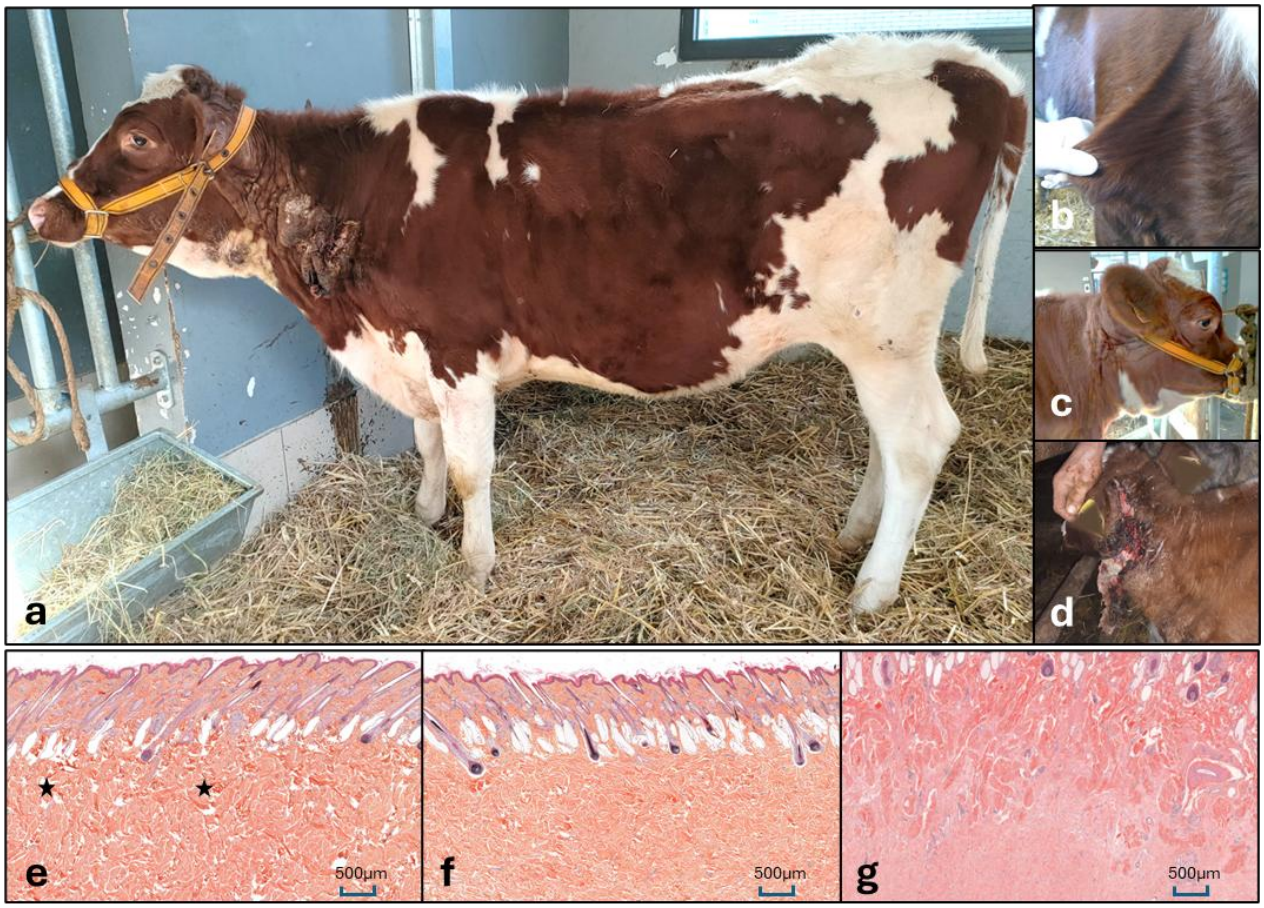

Figure 1. (a-d) Phenotypic and histological features of affected animals. (a) General appearance showing scars and skin folds on the affected animal's neck. (b) Elastic skin easily detached from underlying tissues. (c) Multiple skin folds. (d) Spontaneous injury to the neck. (e-g) Histological analysis of skin samples of affected (e) and control (f) animals showing alteration in the density and size of collagen fibres, with curly fibres surrounded by space (indicated by stars). (g) Fibrous post-lesional skin of an affected animal.

Clinical examination of the three hospitalised animals revealed numerous scars and wounds, along with highly elastic skin that detached easily from underlying tissues. Prominent skin

folds were evident around the neck as well as areas of alopecia, contributing to the impression of excessive skin (Fig.1a–d). Histological analysis demonstrated an altered collagen architecture, with irregular, intensely eosinophilic fibres arranged in a comma-shaped pattern and separated by spaces (indicated by stars) compared with controls (Fig.1e–f). Scar tissue appeared denser and markedly fibrotic, consistent with post-traumatic remodelling (Fig.1g). These clinical and histopathological findings were consistent with the features of classical EDS, except for the absence of joint hypermobility.

All affected animals were daughters of the same AI sire, suggesting a dominant mode of inheritance, with possible germline mosaicism in the sire. Assuming this hypothesis, TDT mapping on paternal phases of 16 affected and 113 healthy daughters enabled us to map the locus on bovine chromosome 11, within a 95% confidence interval ranging from 92,199,563 to 105,923,613 base pairs (bp).

The whole-genome sequence of an affected animal revealed ten private heterozygous variants within the mapped interval, none of which were present in 1,367 control genomes.

Of these, only one variant was predicted to have a protein-altering effect: a nonsense mutation (g.105400157 G>T) located within a strong functional candidate gene for classical EDS, ***COL5A1***, which encodes the Collagen Type V Alpha 1 protein (Fig. 2).

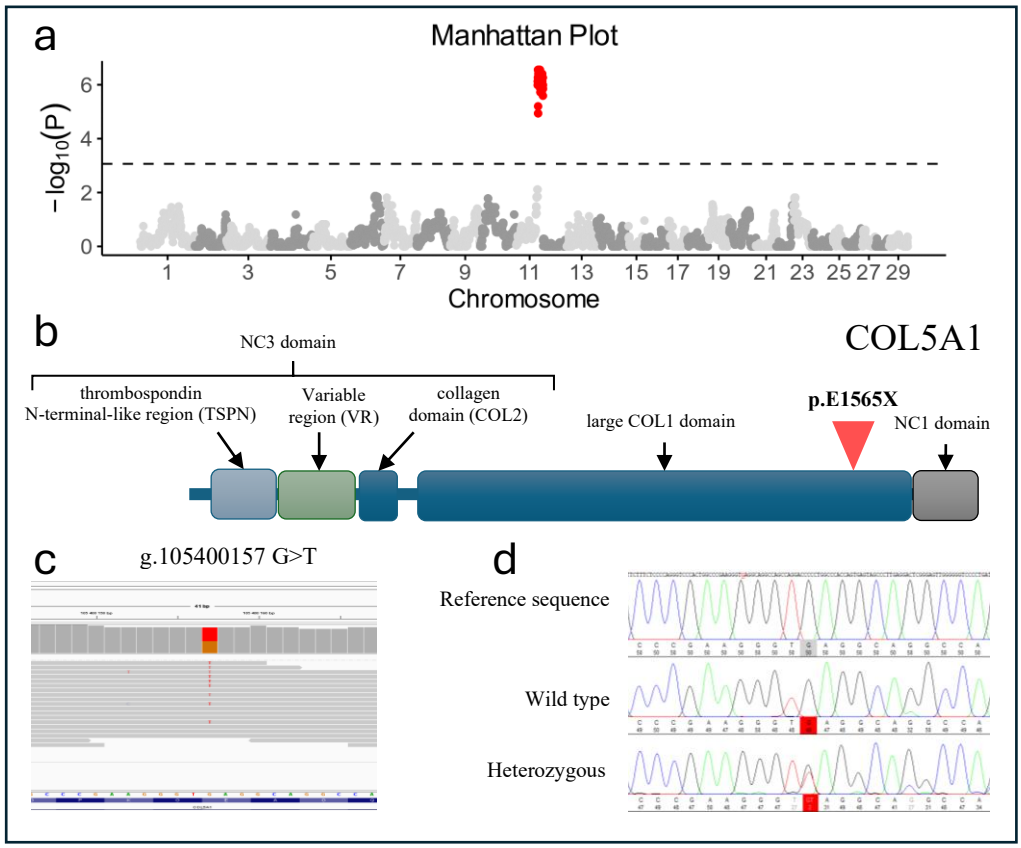

Figure 2. Mapping and identification of the candidate mutation. (a) Manhattan plot of TDT mapping results, showing a significant peak on chromosome 11. The dashed line corresponds to a type I error threshold of 0.05 after Bonferroni correction for multiple testing, considering only two independent tests per chromosome (i.e. -log10(0.05/29/2)). (b) Schematic representation of the COL5A1 protein and its main functional domains, with the position of the mutation indicated. (c) IGV view of the whole-genome sequence at the SNP position. (d) PCR-Sanger genotyping validation: healthy animals match the reference sequence, while affected animals carry the mutation in a heterozygous state.

Subsequent PCR and Sanger sequencing confirmed the *de novo* origin and likely pathogenicity of the ***COL5A1*** variant. This nonsense mutation occurs in exon 61 of 66 and is predicted to generate a truncated protein (p.E1565X) lacking part of the triple helix domain and the entire C-terminal domain, both critical for collagen trimerization. This likely prevents proper protein assembly, impairing interaction with type I collagen fibres and disrupting fibril formation. As a result, the connective tissue in affected animals is expected to be weaker and less cohesive than normal.

The proportion of mutant spermatozoa in the sire was estimated from the genotypes of 113 control descendants. Among these, 39 individuals carried the EDS-associated haplotype in its presumed ancestral form (i.e., without the *de novo* mutation), while 74 individuals carried the alternative paternal haplotype. Using this 39:74 ratio, the proportion of mutant spermatozoa was calculated as 24% [(74 − 39) / (2 × 74)] corresponding to an estimated 48% of mutant germ cells.

Conclusions

In conclusion, we report the first identification of a *COL5A1* mutation associated with Ehlers–Danlos syndrome in cattle, displaying clinical features closely resembling those previously described in some dogs (Bullock *et al.,* 2024). Genetic counselling and expert recommendations were provided, which in this case led to the removal of the bull from the breeding catalogue.

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