# *ITGA6* homozygous splice-site mutation causes junctional epidermolysis bullosa in Charolais cattle

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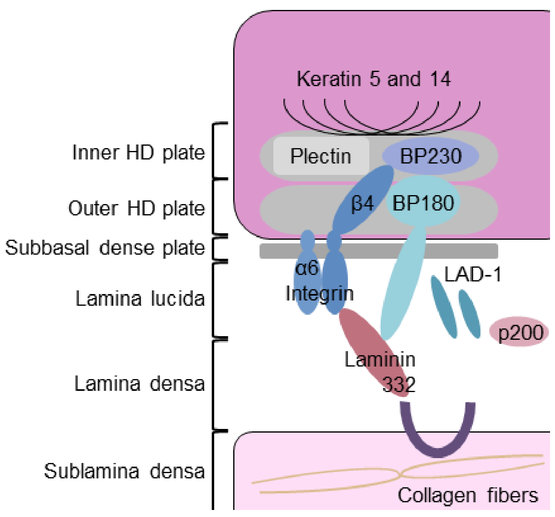
## Introduction

Junctional epidermolysis bullosa (JEB) is a painful and life threatening recessive genetic disorders described in different animal species. It is characterized by mechanically induced blisters of the skin and mucous membranes, with tissue cleavage occurring in the lamina lucida layer of the epidermal basement membrane.




Structure of the HD and the dermo-epidermal zone

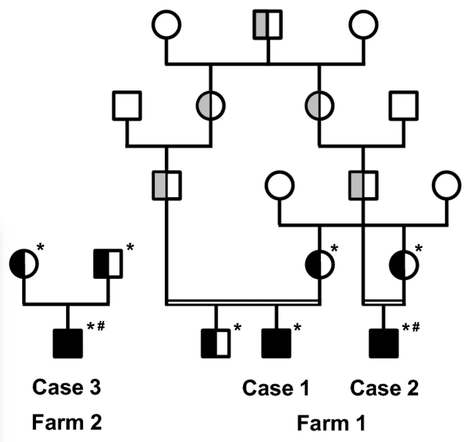
Mutations in several genes encoding components of the hemidesmosome anchoring complex (HD) have previously been associated with this genetic defect.

The aim of this study was to investigate discordant cases of epidermolysis bullosa that did not carry the large deletion within the *ITGB4 gene* that we have previously reported in Charolais cattle.

## Animals and pathological examinations

Three Charolais calves were referred to the French National Observatory for Bovine Abnormalities (www.onab.fr) for congenital skin fragility. These animals, born to unaffected parents in two different herds, were euthanized in their first week of life because of poor prognosis. Pathological examination revealed skin erosion and ulceration in areas of skin folds and friction (c), signs of dysungulation (a), and mucosal blistering (b). These features were similar but milder than those seen in recessive JEB associated with *ITGB4* mutation.

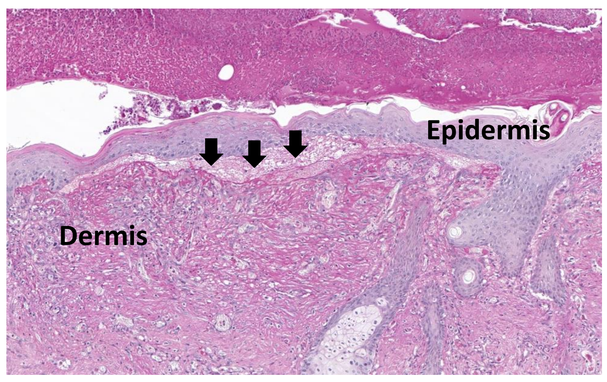

a) Dysungulation
b) Mucosal blistering
c) Skin erosion and ulceration


*Animals with DNA samples
#Individuals selected for whole genome sequencing

## Confirmation of the diagnosis of recessive JEB

Histopathological examination of the lesions showed sub-epithelial splitting between the basal plate of the epidermis and the basement membrane, and inflammatory infiltrates (arrows).




Histological section of the skin at the level of the lesion stained with periodic acid-Schiff (case #2)

## Identification of a candidate variant in the *ITGA6* gene

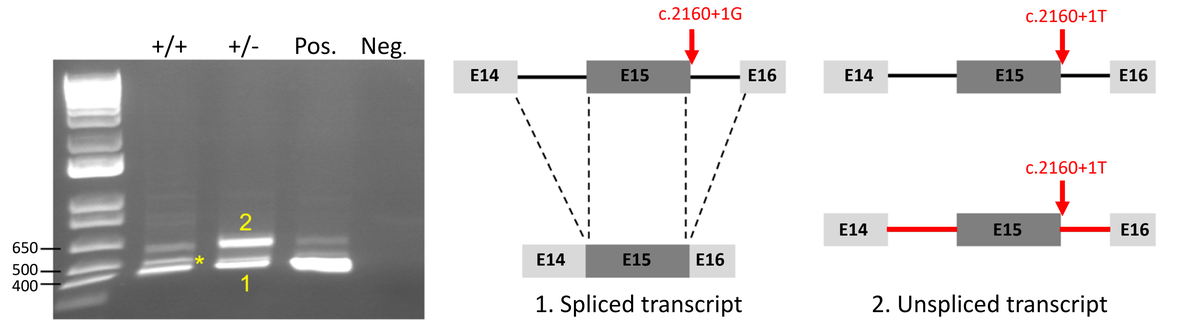
Assuming a recessive inheritance, we mapped the locus on a 6.3-Mb interval on BTA2 and identified a splice donor site mutation in *ITGA6* (c.2160+1G>T; Chr2 g.24112740C>A) as a compelling candidate variant after analysing whole genome sequences of two cases and over 5000 controls. Genotyping this variant by PCR and Sanger sequencing within the pedigree showed a perfect genotype/phenotype status correlation.

## Large-scale genotyping

For further investigations, we added this variant into the EuroGMD SNP array, which is used for genomic evaluation in France, and genotyped 186,154 animals from 15 breeds. This variant segregate at a low frequency of 0.4% in the Charolais natural mating population (n=487), and was absent from the Charolais AI population (n=5765) and from all the other breeds.

## Molecular consequences of the *ITGA6* variant

To investigate the effect of the candidate variant on *ITGA6* splicing we performed RT-PCR analyses and observed an increased retention of introns 14 and 15 in a heterozygous mutant cow as compared to a matched control.




Left) Agarose gel electrophoresis after RT-PCR on an homozygote wild type +/+, one heterozygous carrier +/- and controls (Neg. Water; Pos. Commercial bovine muscle RNA). * third band corresponding to partially spliced transcripts (incomplete excision of intron 14 or 15)

Right) Amplified segments controlled by Sanger sequencing (boxes corresponding to exons)

The mutant mRNA is predicted to cause a frameshift (*ITGA6* p.I657Mfs1), resulting in a premature termination codon. The resulting protein would lack 40% of the amino acid sequence, including the transmembrane domain and half of the integrin alpha 2 domain, which are essential for the proper assembly of the integrin α6β4 dimer and its correct anchoring to the cell membrane. This dimer is a key component of the HD that ensures the attachment of basal epithelial cells to the basement membrane. Several mutations in the 6 proteins of this complex have been reported to cause JEB in humans and mice, but no ITGA6 mutation has been reported in non-model species.

## Conclusion

We report the first evidence of an *ITGA6* mutation causing JEB in livestock and provide a rare example of two recessive genetic defects in a single breed showing similar pathological signs due to mutations affecting two members of the same protein dimer.