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INCIDENCE OF JUNCTIONAL EPIDERMOLYSIS BULLOSA AMONG ROMANIAN DRAFT HORSES

INCIDENȚA EPIDERMOLIZEI JONCȚIONALE LA CAII DIN RASA SEMIGREU ROMÂNESC

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Junctional Epidermolysis Bullosa (JEB) is an inherited disease which causes skin lesions in newborn foals and results in large areas of skin loss. The mutation responsible for the disease is a cytosine insertion in the LAMC2 gene, which results in absent expression of the laminin γ2 polypeptide chain of laminin 5. JEB is inherited as an autosomal recessive trait (Spirito et al. 2002, Milenkovic et al. 2003). Our objective was to analyze Romanian Draft Horses using a set of primers which amplify a fragment from the LAMC2 gene possibly containing the insertion for correctly identifying the normal homozygous and heterozygous carrier horses for the JEB trait. The number of allele peaks depends on whether the horse tested is a heterozygote (carrier) or homozygote (normal or JEB affected). Results suggest that the genetic test will be useful in identifying horses which are heterozygous for the JEB trait and foals with JEB.

Key words: Romanian Draft Horse, JEB, carrier, diagnostication.

Introduction

Epidermolysis Bullosa (EB) is a heterogeneous group of mechano-bullous disorders characterized by fragility of the skin and the mucous membranes. The junctional form of EB, JEB, is characterized by blister formation within the lamina lucida of the basement membrane zone and by an autosomal recessive pattern of inheritance. In the severe Herlitz variant, H-JEB, tissue cleavage results from the mutations in one of the three genes (*LAMA3*, *LAMB3* or *LAMC2*) (*Aumailley et al*. 1998, *Pulkkinen et al.* 1999). These are encoding the three subunits (a3, b3 and g2) of the extracellular adhesion ligand laminin 5 associated with the hemidesmosome-anchoring lament complexes. Likewise, cases of EB have been described in different species, such as sheep (*Bruckner-Tuderman et al.* 1991), dogs (*Palazzi et al.* 2000), cats (*Olivry et al.* 1999), and rats (*Brenneman et al.* 2000).

In horses, JEB is an inherited disease that causes skin lesions in newborn foals and results in large areas of skin loss. The disease was first discovered in Belgian draft horses. Different areas of the body were affected, in particular the

limbs with recurrent loss of the hooves. The phenotype of the affected foals suggests a condition in horses similar to H-JEB in humans. Affected foals were born with skin blistering, skin and buccal ulceration followed by the loss of the hooves, as ascertained by a veterinarian and confirmed by histological examination (*Milenkovic et al.* 2003). The affected skin showed disjunction of the epidermis from the underlying dermis at the dermal-epidermal junction.

In France, a type of lethal junctional EB was described for the first time in the Trait Breton and Trait Comtois draft horses and the limited family data are compatible with a recessive mode of inheritance (*Goureau et al.* 1989).

The mutation associated with the clinical signs of JEB in Belgian and French draft horses has been identified and is linked to the γ2 subunit of the laminin-5 gene. The mutation is a cytosine insertion in the genomic nucleic acid sequence of affected horses at position 1368 of the laminin γ2 encoding polynucleotide, a frame shift, and a premature termination codon. This results in an absent expression of the laminin γ2 polypeptide. An autosomal recessive mode of inheritance of this mutation has been verified (*Spirito et al.* 2002, *Milenkovic et al.* 2003).

Material and Methods

In our research we analyzed a population of Romanian Draft Horses. The isolation of genomic DNA from fresh blood was performed with Wizard Genomic DNA Extraction Kit (Promega).

We used one set of primers, which amplify a fragment from the LAMC2 gene possibly containing the insertion. The forward primer was labeled with 6-FAM dye. PCR was performed in a GeneAmp 9700 PCR System (AppliedBiosystems). The reactions were carried out in 25-µl final volume containing PCR Buffer, $MgCl_2$, 200 µM of each dNTP, 0.5 µM of each primer, 0.5 units of AmpliTaq Gold DNA Polymerase, diluted DNA (50 ng per reaction) and nuclease-free water. PCR amplifications were performed in 0.2 ml tubes using 30 cycles with denaturation at 95 ºC (30 s), annealing at 57 °C (30 s) and extension at 72 °C (45 s). The first denaturation step was performed at 95 ºC (10 min) and the last extension took 10 min. at 72 °C.

PCR products were loaded with the GeneScan-500 ROX Internal Size Standard (AppliedBiosystems) into one of the ABI PRISM 310 DNA Genetic Analyzer (AppliedBiosystems).

The results were analyzed with the GeneScan 3.1.2. Software (AppliedBiosystems) which assigns a base pair size for each signal. GeneScan data can then be exported directly to Genotyper 2.5.2. Software (AppliedBiosystems) for automated genotyping.

Results and Discussions

Our objective was to analyze Romanian Draft Horses using a set of primers which amplify a fragment from the LAMC2 gene possibly containing the insertion, for correctly identifying the normal homozygous and heterozygous carrier horses for the JEB trait.

PCR was performed on DNA samples with fluorescently labeled primers designed to amplify the region containing the mutation. The mutation is a single base insertion, and thus, carriers have a PCR product that is one base longer than the normal allele. The single base difference is detected by analysis of the PCR products on an ABI 310 DNA Genetic Analyzer.

An amplified fragment for a normal horse had 169 bp. The conditions for PCR were selected so that the two primers could amplify the DNA from normal, carrier and JEB affected horses.

In our experiment successful amplification yields one or two allele peaks with an expected size of 169 and (or) 170 bp. The number of allele peaks depends on whether the individual tested is a heterozygote (carrier) or homozygote (normal or JEB affected). If we test a normal horse we must obtain just one peak at 169 bp. If we analyze a homozygous affected horse we also obtain just one peak, but at 170 bp. If the horse is a heterozygous carrier we must obtain two peaks at 169 and 170 bp because one allele is normal and the other one contains the insertion.

We did not find any carrier or affected horses among the Romanian Draft Horse population analyzed.

In Figure 1 the profile for a heterozygous carrier and for a homozygous normal horse are shown.

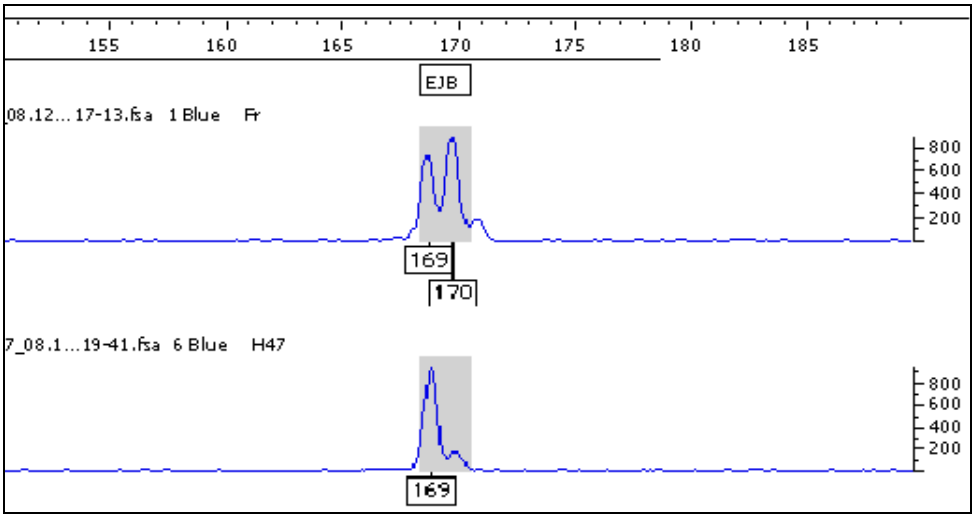

Figure 1: Genotyper software analysis of PCR amplification product for a heterozygous carrier and for a normal homozygous horse.

Conclusions

The identification of the causal mutation of JEB is of great importance to draft horse breeders. A rapid, simple genotyping method by DNA amplification from blood or hair samples detecting normal and mutated fragments is now available for genetic tests. The identification of healthy carriers for the mutation allows the development of different breeding strategies. Population reproductions can be conducted, avoiding mating between carriers to obtain unaffected foals. Alternatively, breeder associations may decide to eradicate the mutation by preventing all carriers to reproduce.

In conclusion, this study proposes a rapid molecular test for the identification of healthy carriers which will help the breeders to eliminate the disorder from their populations.

Consequently, the investigation of possible occurrences of the affected gene among other draft horse populations from other countries and among other breeds will be of interest.

Bibliography

1. **Aumailley M., N., Smyth** (1998) - *The role of laminins in basement membrane function.* J. Anat. 193, 1-21.

2. **Brenneman K.A., T., Olivry, D.C., Dorman** (2000) - *Rudimentary hemidesmosome formation in congenital generalized junctional epidermolysis bullosa in the Sprague-Dawley rat.* Vet. Pathol. 37, 4336-4339.

3. **Bruckner-Tuderman L., F., Guscetti, F., Ehrensperger** (1991) - *Animal model for dermolytic mechanobullous disease: sheep with recessive dystrophic epidermolysis bullosa lack collagen VII.* Journal of Invest. Dermatol. 96, 452-458.

4. **Spirito F., A., Charlesworth, K., Linder** (2002) - *Animal models for skin blistering conditions: absence of laminin 5 causes hereditary junctional mechanobullous disease in the Belgian horse.* J. Invest Dermatol. 119, 684-691.

5. **Milenkovic D., S., Chaffaux, S., Taourit, G., Guérin** (2003) - *A mutation in the LAMC2 gene causes the Herlitz junctional epidermolysis bullosa (H-JEB) in two French draft horse breeds.* Genet. Sel. Evol. 35, 249-256.

6. **Goureau J.M., C., Feillou, M., Morand, J.F., Courreau, A.M., Dupere, A., Alliot** (1989) - *Épidermolyse bulleuse jonctionelle léthale chez le cheval de trait en France.* Bull. Acad. Vét. Fr. 62, 345-353.

7. **Olivry T., S.M., Dunston, M.P., Marinkovich** (1999) - *Reduced anchoring fibril formation and collagen VII immunoreactivity in feline dystrophic epidermolysis bullosa.* Vet. Pathol. 36, 616-618.

8. **Palazzi X., T., Marchal, L., Chabanne, A., Spadafora, J.P., Magnol, G., Memeguzzi** (2000) - *Inherited dystrophic epidermolysis bullosa in inbred dogs-A spontaneous animal model for somatic gene therapy.* J. I.. Dermatol. 115, 135-137.

9. **Pulkkinen L., J., Uitto** (1999) - *Mutation analysis and molecular genetics of epidermolysis bullosa.* Matrix Biol. 18, 29-42.