

Junctional Epidermolysis Bullosa in Belgian Draft Horses in North America: Results of Genetic Testing (2002–2012)

John D. Baird, BVSc, PhD*; Lee V. Millon, BS; and M. Cecilia T. Penedo, PhD

Junctional epidermolysis bullosa (EB) in the Belgian draft horse and other draft horse breeds is a genodermatosis inherited as an autosomal recessive trait. The mutation responsible is a cytosine insertion (1368 insC) in the *LAMC2* gene, which results in the absent expression of the laminin α -2 polypeptide chain of the heterotrimer laminin-332 (previously laminin-5). A polymerase chain reaction (PCR) test was developed to identify carriers of the mutation through the use of mane hairs. Mandatory testing of breeding stallions was instituted by the Belgian Draft Horse Corporation of America on November 1, 2002, and by the Canadian Belgian Horse Association on January 1, 2003. In the following 10 years (until December 31, 2012), a total of 2176 registered Belgian draft horse stallions have been tested. The number of stallions identified as carriers of the *LAMC2* mutation was 319 (12.5%). The genetic testing of breeding mares for the *LAMC2* mutation has been on a voluntary basis. Over the first 10 years of genetic testing, there has been no statistically significant change in the percentage of carriers of the *LAMC2* mutation. Authors' addresses: Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, ON N1G 2W1 Canada (Baird); Veterinary Genetics Laboratory, University of California, One Shields Avenue, Davis, CA 95616 (Millon, Penedo) e-mail: jbaird@ovc.uoguelph.ca. *Corresponding and presenting author. © 2013 AAEP.

1. Introduction

Epidermolysis bullosa (EB) is an inherited mechanobullous disorder characterized by skin fragility and blister formation after minor trauma or traction on the skin.¹ There are many clinically distinctive phenotypes, all of which have skin blistering as a major feature, but variable risks of extracutaneous manifestations and premature death. In humans, more than 1000 different mutations involving 14 structural genes within the skin have been documented to lead to the clinical phenotypes of EB.^{1,2} Mutations result in either abnormal, absent, or significantly reduced levels of a specific protein that is

important in epidermis to dermis adhesion. The result is shearing of the skin or blistering with ultrastructurally uniform cleavage planes.³

EB is currently classified into four major types, on the basis of the level of the skin where the missing or abnormal structural skin protein is located and the corresponding ultrastructural level of cleavage.^{1,3} In EB simplex, cleavage and blister formation occurs within the epidermis. In junctional EB (JEB), blister formation occurs within the lamina lucida, an electron-lucent region that contains anchoring filaments that connect the basal keratinocytes to the underlying lamina densa. In dystrophic EB, blis-

NOTES



Fig. 1. Newborn Belgian foal shows characteristic skin lesions of junctional epidermolysis bullosa-Herlitz form.

tering occurs in the dermis (or sublamina densa). The fourth type of EB is the Kindler syndrome, which is a mixed type of EB that exhibits multiple cleavage planes within the affected skin.^{1,3}

JEB-Herlitz (JEB-H) represents the most severe form of EB, which is characterized by generalized, extensive mucocutaneous blistering at birth with erosions of the skin and mucous membranes, and dental enamel hypoplasia.^{3,4} In humans, the disease is lethal in early childhood.¹ JEB-H is most often caused by homozygous null mutations in the genes *LAMA3*, *LAMB3*, or *LAMC2*, each gene encoding for one of the three chains of the heterotrimer laminin-332 (previously laminin-5).³ The Herlitz form of JEB is the result of complete absence of laminin-332.^{4,5}

Laminin-332 is a major adhesion protein within the basement membrane zone of the skin and mucous epithelia that provides stable anchorage of basal epithelial cells (keratinocytes) to the underlying dermis by connecting the hemidesmosomal component $\alpha6\beta4$ integrin to collagen VII-containing anchoring fibrils.⁴ Laminin-332 is an essential component of the dermal-epidermal basement membrane.⁵ The usual mode of transmission is autosomal recessive.^{3,4}

In North America, reports of a junctional mechanobullous disease in Belgian foals were first published in the late 1980s.⁶⁻⁸ The most consistent skin changes are irregular, round, red, and ulcerated areas over the bony prominences of the hocks, stifles, hips, carpi, elbows, and fetlocks (Fig. 1). The severity and extent of the skin lesions progress with age. Affected foals usually have very extensive oral erosions and ulcers, especially around the base of the incisor teeth. One of the most characteristic findings is that the temporary incisor teeth, which are not usually noticed until 8 to 14 days of age, are visible at birth. The teeth are very white and have irregular serrated edges, with pitted enamel. Excessive amounts of blood-tinged saliva may occur as a result of oral ulceration. Irregular

areas of ulcers are along the coronary bands, which may progress to sloughing of the hoof.⁶⁻⁹

The clinical, histopathological, ultrastructural, and immunohistochemical findings have shown that the disease in North American Belgian draft foals fits all the criteria of the JEB-H form of EB.¹⁰ The mutation responsible for this particular form in Belgian draft horses was first identified on January 24, 2001, by researchers at the INSERM 634 Laboratory, University of Nice, France.¹⁰ The mutation is a cytosine insertion in the genomic nucleic acid sequence of affected horses at position 1368 of the laminin $\alpha2$ -encoding polynucleotide, resulting in a frame shift that leads to a premature termination codon and absent expression of the *LAMC2* gene.¹⁰ An autosomal recessive mode of inheritance of this mutation was verified.¹⁰

After the identification of the *LAMC2* mutation, a commercial polymerase chain reaction (PCR) test was developed at the Veterinary Genetics Laboratory (VGL), School of Veterinary Medicine, University of California-Davis. This test was performed on DNA samples with fluorescence-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 by capillary electrophoresis on ABI 3730 DNA sequencer.⁹

The availability of a commercial PCR test and appropriate genetic counseling made it possible for Belgian draft horse breeders to avoid the financial and genetic losses associated with the birth of JEB-H foals. Commencing on November 1, 2002, the Belgian Draft Horse Corporation of America (BDHCA) instituted rules that require the sire of a foal being registered to have been DNA-profiled and JEB-tested. This testing program is organized by the corporation office. The breed association provides hair sample collection information and forms to the owners of horses to be tested. Samples are submitted to the VGL for testing, and results are reported directly to the breed association office. The JEB results are printed on the Certificate of Registry. The Canadian Belgian Horse Association (CBHA) instituted the same requirements that commenced on January 1, 2003. The testing of mares for the *LAMC2* mutation has been on a voluntary basis; however, when mares are bred by artificial insemination with frozen semen they are required to be DNA- and JEB-tested. Foals resulting from frozen semen insemination are also required to be DNA- and JEB-tested. The aim of the genetic testing program is (1) to prevent the birth of JEB-H Belgian foals and (2) to reduce the number of carrier animals in the population.

2. Materials and Methods

Until December 31, 2012, mane hair root samples from 1785 stallions, 301 mares, and nine geldings

registered with the BDHCA and from 391 stallions, 65 mares and three geldings registered with the CBHA were forwarded to the VGL for the commercial PCR test for the *LAMC2* mutation. Logistic regression models were used to examine the relation between year of testing and *LAMC2* mutation carrier status, the binary outcome (SAS 9.2). We also allowed for quadratic effect, breed registry, and interactions.

3. Results and Discussion

In the BDHCA registry, 206 (11.5%) of 1785 stallions tested between 2002 and 2012 were found to be carriers of the *LAMC2* mutation. In the CBHA registry 47 (12.0%) of 391 stallions tested between 2003 and 2012 were found to be carriers of the *LAMC2* mutation. Over the same time period, the number of mares JEB-tested has been low. In both registries, only 336 mares have been tested, with 65 (17.8%) identified as carriers of the *LAMC2* mutation. In the past 3 years (2009–2012) in the BDHCA registry, of the 42 horses found to be carriers, 21 (50%) were from mating in which the stallion was a JEB-tested non-carrier and the mare had not been JEB-tested.

On statistical analysis, there were no quadratic effects or interactions (P values >0.25). There was also no effect of breed registry (P value 0.8149) (odds ratio = 1.041, 95% confidence interval = 0.743, 1.459) and no trend over years of testing (P value 0.2339) (slope = -0.275 , 95% confidence interval = -0.0729 , 0.0178). There has been no statistically significant difference in the *LAMC2* mutation carrier rate in the Belgian stallion population in both the BCDHA and CBHA registries since testing over the 10-year period of testing.

There is anecdotal evidence from equine practitioners, the breed association offices, and breeders that the occurrence of JEB-H foals has declined markedly. The last two cases of JEB-H that the authors are aware of were born in Indiana in 2008 and in Ontario in 2009. The lack of JEB-H foals has been

attributed to Belgian draft horse breeders selecting sires that are not carriers of the *LAMC2* mutation. In 2012, of 132 animals that were JEB-tested 12 (10 stallions, two mares) were identified as carriers. Of the 132 tested animals, 100 (75.8%) were sired by non-carrier stallions, 30 (22.7%) were sired by stallions that were born before the introduction of mandatory testing (not JEB-tested), and two were sired by one known carrier stallion.

Acknowledgments

The authors would like to thank William Sears, Department of Population Medicine, University of Guelph, for statistical assistance and the secretaries of the Belgian Draft Horse Corporation of America and the Canadian Belgian Horse Association for their access to breed registration data.

References

1. Gonzalez ME. Evaluation and treatment of the newborn with epidermolysis bullosa. *Semin Perinatol* 2013;37:32–39.
2. Fine JD. Inherited epidermolysis bullosa: recent basic and clinical advances. *Curr Opin Pediatr* 2010;22:453–458.
3. Fine JD, Eady RAJ, Bauer EA, et al. The classification of inherited epidermolysis bullosa (EB): report of the Third International Consensus Meeting on Diagnosis and Classification of EB. *J Am Acad Dermatol* 2008;58:931–950.
4. Laimer M, Lanschuetzer CM, Diem A, et al. Herlitz junctional epidermolysis bullosa. *Dermatol Clin* 2010;28:55–60.
5. Kiritsi D, Has C, Bruckner-Tuderman L. Laminin 332 in junctional epidermolysis bullosa. *Cell Adh Migr* 2013;7:135–141.
6. Frame SR, Harrington DD, Fessler J, et al. Hereditary junctional mechanobullous disease in a foal. *J Am Vet Med Assoc* 1988;193:1420–1424.
7. Johnson GC, Kohn CW, Johnson CW, et al. Ultrastructure of junctional epidermolysis bullosa in Belgian foals. *J Comp Pathol* 1988;98:329–336.
8. Kohn CW, Johnson GC, Garry F, et al. Mechanobullous disease in two Belgian foals. *Equine Vet J* 1989;21:297–301.
9. Baird JD, Millon LV, Dileanis S, et al. Junctional epidermolysis bullosa in Belgian draft horses, in *Proceedings*. Am Assoc Equine Pract 2003;49:122–126.
10. Spirito F, Charlesworth A, Linder K, et al. Animal models for skin blistering conditions: absence of laminin 5 causes hereditary junctional mechanobullous disease in the Belgian horse. *J Invest Dermatol* 2002;119:684–691.