

Genetics of Skin Disease in Horses



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KEYWORDS

- Genetics • Hereditary skin disorders • Horses • Insect bite hypersensitivity
- Melanoma • Ehlers-Danlos syndrome • Chronic progressive lymphedema

KEY POINTS

- Genetic testing can be used as an instrument for breeding against hereditary genetic skin diseases in horses.
- Genetic tests are available for hereditary equine regional dermal asthenia, warmblood fragile foal syndrome, junctional epidermolysis bullosa, incontinentia pigmenti, and hypotrichosis.
- Insect bite hypersensitivity is a common complex disease affected by several genes (polygenic inheritance) and environmental factors.
- Genomic technologies emerge as a useful tool to understand the genetics behind common complex skin diseases.
- Horse can serve as an animal model for the human skin conditions.

INTRODUCTION

Structure and Function of the Skin

Skin displays an astonishing diverse set of phenotypes and physiologic functions ranging from skin and hair color to immune function. Acting as a primary barrier between the body and its environment, its key functions are (1) to prevent environmental compounds from permeating into the epidermal and dermal layers, giving rise to an immune response and (2) to restrain excessive water passage from one side to the other of the epidermis, in particular providing an effective barrier to the loss of water. Skin functions as an immunologic barrier and is often the first organ to connect to adverse allergens. It is an immunologic organ, nominated as a peripheral lymphoid organ that contains several important types of immunocompetent cells in both epidermis and dermis.^{1–3} The immune competence in epidermis is mainly mediated

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by keratinocytes secreting immunoregulatory compounds and antigen-presenting Langerhans cells, whereas in the deeper layer dermis cells such as assorted dendritic cells, mast cells, and T-cell subsets play a crucial role.¹ As a group, these well-organized cells mediate cutaneous immunosurveillance. It can be expected that immune reactions in the skin are equally important to those occurring within classical lymphoid organs in protection from harmful foreign substances. Further, skin provides sensory perception for touch and pain, temperature regulation, and pigmentation and produces structures such as hair and hoof.

Mammal skin is a multicellular organ, and its *morphogenesis* includes expression of multiple genes in a coordinated fashion. The anatomic structures of equine skin have been reported in multiple studies⁴⁻⁷ (Fig. 1). Skin is generally composed of 2 layers, the outer nonvascular epidermis and the inner vascular and sensitive dermis. In horses, the epidermis consists of 5 to 7 cell layers, excluding the horny layer in haired body skin, that are constantly multiplying from its deeper layers and eventually drop off at the surface.^{6,8} The most abundant cell type of the epidermis is keratinocytes (about 85%).^{6,9} The dermis is mostly composed of connective tissue and account for most of the strength and elasticity of the skin. It composes an interconnected mesh of elastin and collagenous fibers, produced by fibroblasts. Normally the dermis is sparsely populated with cells; however, fibroblasts, dermal dendrocytes, and mast cells are present throughout with varying density.^{5,8,10} Melanocytes are most commonly present in the basal layer of epidermis; however, these cells can also be present in the superficial layers of dermis in strongly pigmented skin. The dermis also supports and maintains many other structures, such as blood and lymph vessels, hair follicles, muscles, nerves, and glands (sweat and sebaceous). In horses, the general skin thickness varies over the body (eg, thickest on the forehead, dorsal neck, dorsal thorax, rump, and base of the tail),⁸ with the average thickness of the mane and tail epidermis being 91 μm versus 53 μm for the epidermis of the general body skin under the coat.⁸

Under normal circumstances, hair growth occurs in a cycle, rather than continuous growth.¹¹ Hair follicles are the structures that contain the roots of the hair and consist of 5 major components: the dermal hair papilla, the hair matrix, the inner root sheath, the outer root sheath, and the hair shaft itself.¹² There are 3 main phases of the hair growth cycle: anagen, catagen, and telogen. Anagen is the growing stage where

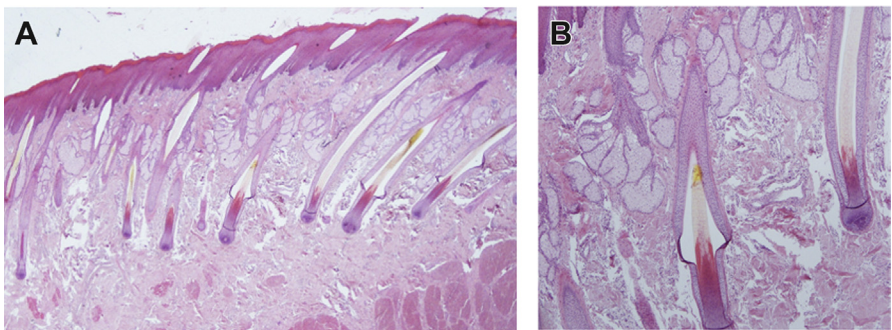


Fig. 1. Lip skin biopsy from a healthy horse. (A) Skin structure: epidermis and dermis including hair follicles (light microscopy X1). (B) Zoom of the dermis hair follicles (light microscopy X4). (Courtesy of Eva Hellmén, DVM, Professor, Dipl. ECVP. Swedish University of Agricultural Sciences, Department of Anatomy, Physiology and Biochemistry, Uppsala, Sweden.)

hair is produced by mitosis in cells of the dermal papilla. Catagen is the transition stage where a constriction of the hair bulb takes place and the distal follicle becomes broad and presses the hair outward. Telogen is the resting stage where a secondary germ is formed. The hair growth continues until it attains its predetermined length, a mechanism that is under genetic regulation. Knowledge of the genetic factors that regulate the hair cycle is limited. Equine mane hairs are similar to human scalp hairs because they grow to a greater length than the body hairs, that is, mane hairs have a long anagen growth phase. Periodic molting of hairs allows the pelage to adapt to seasonal changes. Shedding is predominantly influenced by photoperiod and affects the hairs of the body, whereas hairs of the mane, tail, and fetlock are basically exempt from such regulation.¹²

Role of Genetics in Skin Morphogenesis and Disease

The process of generating the anatomic structures of the equine skin has, to the authors' knowledge, not been reported. However, information on skin morphogenesis in other domestic mammals and human is available.¹² In mammals, distinct signaling patterns specify different developmental stages that ensure correct morphogenesis of skin and its associated structures such as hair and hoof.^{13,14} The whole process requires a tightly controlled sequence of signaling events. Because genetics regulate organ development, and adult phenotypes are usually defined during development, investigations on differences in gene expression, contribution of new genes, changes in alternative splicing, and role of regulatory RNAs (long noncoding RNA [lncRNA] and microRNA [miRNA] [Box 1]) are important to understand phenotypic diversity such as disease. Genetic regulation of mammalian organ development/morphogenesis is only partly understood.^{15–17} For instance, the contribution of lncRNAs to organ development remains vastly unexplored in mammals, including horses.

Since the onset of domestication, horses have been strongly selected for speed, strength, gaits, and endurance-exercise traits. The development of specific horse breeds has resulted in the selection for athletic phenotypes that enable the use of horses for riding, racing, and recreation, with clear differences in morphology and behavior. The breed formation process has also enriched different horse breeds for

Box 1

Definitions of biological terminologies

Morphogenesis: the developmental process of the shape/morphology of an organism.

lncRNA: long (200 bp) noncoding RNAs are molecules that are not translated to proteins. They play a role in *the regulation of gene expression*.

miRNA: micro (~20 bp) RNA are shorter than lncRNA but also function in regulating gene expression.

Genome-to-phenome research: the study of the link between the DNA sequence (genome) and the different phenotypic characteristics (phenome) of an organism.

GWAS: a genome-wide association study is an approach used to investigate the association of a specific trait (a phenotype such as a shape or disease) to its causative genetic variants.

SNP: a single nucleotide that is polymorphic in a population.

Epistasis: is when the effect of one gene depends on the effect of another gene.

Breeding value: the value of an individual animal in a breeding program for a specific trait. An animal's expected breeding value is the sum of the halves of the breeding values of each parent.

disease mutations, and sometimes these mutations are breed specific. Disease mutations in horses offer the advantages of spontaneous disease models.

This review on equine genetic skin disease aims to present some of the latest advances in *genome-to-phenome* research in a nonmodel species of both medical and agricultural interest. It will summarize equine skin disease for which genetic tests are available and discuss their potential role as a complementing diagnostic tool. Genetic testing as an instrument for breeding against genetic skin diseases in horses will also be outlined. The review ends with perspectives on future studies in relation to these skin diseases.

Insect Bite Hypersensitivity

Insect bite hypersensitivity (IBH) is the most common allergic skin diseases in horses worldwide. The allergic reaction is toward the biting midges *Culicoides* spp, but other insects can also be involved or be cross-reactive.¹⁸ The acute stage of the disease involves an immunoglobulin E (IgE)-mediated type I hypersensitivity reaction, but a delayed type IV hypersensitivity reaction is likely present during the chronic stages of the disease. The clinical signs are gradual in onset and seasonally recurrent because the insects only live during the warm period from spring to autumn. The symptoms are severe pruritus in the mane and tail, leading to self-excoriations and secondary changes in the skin including crusted papules, hyperkeratosis, lichenification with thickening of the skin, diffuse to complete alopecia, and ulcerations (Fig. 2). The lesions can also be seen in the face and ears, at the chest and/or ventrum, in the axillae, and at the hips.¹⁹

All breeds can develop IBH and the prevalence has been calculated as a wide range, between 3% and 60% in different studies.^{20–23} The Icelandic horse is particularly affected because the midges do not exist in Iceland and the horses get sensitized when exported abroad. The higher prevalence for Icelandic horses exported to the continent has been demonstrated in several studies and shows that the exposure to the midges is essential for development of IBH. It also shows that the prevalence for Icelandic horses born outside Iceland does not differ very much from other breeds, with figures between 6.7% and 8%.^{20,22} The heritability in various breeds ranges from 0.16 to 0.30.²⁴ Heritability was estimated to be 0.16 in Friesian horses, 0.24 in Dutch Shetland ponies, and 0.27 in Icelandic horses.^{24–26} When severity was taken into account in the Swedish study of Icelandic horses, heritability was estimated at 0.30. In the same study, offspring from mares with IBH demonstrated a higher risk of developing the disease. The prevalence was in the range of 0% to 30% in different paternal half-sib groups, which clearly shows the difference on an individual level in the heritability of the disease.²⁴

At this time, there are no validated tests for the diagnosis of IBH. Therefore, the diagnosis is clinical and involves ruling out other pruritic diseases such as parasites, together with the typical clinical presentation of seasonal recurrent pruritus in the mane and tail. Intradermal skin tests or serology tests for antigen-specific IgE have been used to identify allergens involved in the allergic reaction, but several studies have shown that the available tests have low sensitivity and/or specificity and are not standardized.^{27,28}

In the 1990s, it was determined using serology that certain major histocompatibility complexes (MHC), that is, equine leukocyte antigen (ELA) specificities, were linked to IBH susceptibility.^{20,29,30} Later, DNA genotyping, as a substitute for ELA serology, showed that the ELA class II region in horses was associated with IBH susceptibility.³¹ The same ELA class II risk factors were shown to contribute to equine IBH in 2 distinct horse breeds, Icelandic horses and Exmoorponies.³¹ Yet another study found



Fig. 2. Clinical signs of IBH. (A, B) Alopecia, broken hairs, and focal ulcerations in the mane. (C, D) Alopecia, hyperpigmentation, and lichenification at the base of the tail and in the mane. (Courtesy of Rebecka Frey, DVM, AniCura Norsholms Djursjukhus, Norsholm, Sweden.)

associations between genetic markers in ELA class II region and IBH susceptibility in Icelandic horses.³² However, this study also identified susceptibility to IBH in 4 candidate allergy-related genes: CD14 receptor (*CD14*), interleukin 23 receptor (*IL23R*), thymic stromal lymphopoietin (*TSLP*), and transforming growth factor beta 3 (*TGFB3*).³² Because the genomic structure of the horse MHC class II region has been resolved,^{33,34} it may now be possible to fine map the responsible susceptibility risk factors. The MHC haplotype diversity was recently determined in Icelandic horses, which should further facilitate gene mapping within the region in this particular breed.³⁵

With the overall goal to both breed against IBH and improve the health and welfare of IBH-affected horses via development of new treatments, several studies had the aim to scan the whole equine genome to identify susceptibility genes. *Genome-wide association studies* (GWAS) using large numbers of *single nucleotide polymorphisms* (SNPs) in cases and control animals have most frequently been used for this purpose. The first GWAS on IBH in horses was performed on Shetland pony mares living in the Netherlands.³⁶ Significant associations to IBH were detected on 12 chromosomes, including ECA20, where ELA is located. Subsequently, several GWAS for IBH have been performed within Icelandic horses, Exmoor ponies, Friesian horses, and Belgian Warmbloodhorses^{37–42} (Table 1). One of these GWAS was performed using both

Method (Array)	Breed	GWAS Windows Hits	Overlapping Windows Hits ^a	ELA	References
Case-control GWAS (70K)	Icelandic horse	20	9	Yes	Schurink et al, ³⁶ 2012
Case-control GWAS (50K)	Icelandic horse	29	6	No	Shrestha et al, ³⁸ 2015
Case-control GWAS (50K & 70K)	Icelandic horse	30	12	No	Shrestha et al, ⁴² 2019
GWAS IgE levels (70K)	Icelandic horse	35	8	Yes	François et al, ⁴¹ 2019
Case-control GWAS (70K)	Shetland pony	20	8	Yes	Schurink et al, ³⁶ 2012
Case-control GWAS (50K)	Shetland pony	18	5	No	Schurink et al, ³⁷ 2013
GWAS IgE levels (70K)	Shetland pony	35	5	Yes	François et al, ⁴¹ 2019
Case-control GWAS (670K)	Exmoor pony	24	4	Yes	Velie et al, ³⁹ 2016
Chi-square test (TaqMan assay)	Exmoor pony	2	2	No	Shrestha et al, ⁴² 2019
Case-control SNP- & CNV-based GWAS (670K)	Friesian horse	23	6	Yes	Schurink et al, ⁴⁰ 2018
GWAS IgE levels (670K)	Belgian Warmblood horse	35	5	No	François et al, ⁴¹ 2019

^a Comparison with recent bibliography up to 2019.^{36–42}

copy number variants (one type of structural variation) and SNPs in the analysis, and both types of genetic markers were able to identify a clear association between the ELA region and IBH susceptibility in Friesian horses.⁴⁰ Recently, in an attempt to improve the power to identify underlying genetic variants for IBH, an objective diagnosis of horses with and without IBH was performed using IgE levels against several recombinant *Culicoides* spp. allergens.⁴¹ The study was performed in Shetland ponies, Icelandic horses, and Belgian Warmblood horses. Several chromosome regions could be detected that confirmed previously IBH-associated regions, but novel regions were also identified. The use of allergen-specific IgE levels as a quantitative phenotype had an added value because a larger number of associations were obtained, as compared with a binary case-control design using the same horse material.

In summary, breed differences in susceptibility to IBH exist, although a common genetic background is present to some extent among certain breeds. The overall conclusions from these genomic studies are that IBH is a complex disease that involves the additive effects of many genes and the interplay between genetic and environmental factors (see [Table 1](#)).

Melanoma and Vitiligo

Melanomas in horses appear as black lumps or nodules most commonly near hairless areas, such as under the tail, around the anus, or in the sheath of geldings. They can

develop anywhere and, even if most of the tumors are benign, they tend to become malignant by aging. The melanomas occur in higher frequency in gray-colored horses, and around 80% of all greying horses will get melanomas by 15 years of age. The diagnosis is made by fine-needle aspiration or biopsy of the nodules. Vitiligo is a disease that leads to depigmentation of the skin due to destruction of melanocytes and as a result the hairs turn white. It presents as small, focal, and often well-circumscribed white spots in the coat or at mucocutaneous junctions. Arabian fading syndrome is a form of vitiligo that develops in young Arabians by 1 to 2 years of age. All colors in the breed can have vitiligo, but it is more common in horses with gray hair coat. It is characterized by round depigmented macules that coalesce to patches, around the eyelids, lips, and muzzle and occasionally around the genitalia. There is no visible inflammation of the skin, and the horse has no other symptoms. The depigmentation can wax and wane in intensity but is usually permanent. The diagnosis is done by clinical picture and biopsy for histopathology.

Melanoma and vitiligo-like depigmentation are among the most common skin diseases in gray horses and they can reach a prevalence between 10% and 80%, depending on the horse's age or breed.^{43–46} Malignancy of melanomas in solid colored horses is more severe than in gray horses,⁴⁷ although metastases in the lymph nodes, liver, spleen, skeletal muscle, lungs, and surrounding or within blood vessels may occur in gray horses.⁴⁸ In 2008, a 4.6 kb duplication in the *STX17* (syntaxin 17) gene was identified as the cause of gray phenotype.⁴⁹ This duplication, therefore, was proposed to be involved in the promotion of melanocyte proliferation by upregulating the expression of *STX17* and/or *NR4A3* (nuclear receptor subfamily 4, group A, member 3) genes. The region contains regulatory elements with melanocyte-specific effects (microphthalmia-associated transcription factors), and higher copy number of the *STX17* duplication is present in aggressive tumors of gray horses.^{50,51} However, the mechanistic features of the duplication remain unknown. Moreover, *RACK1* (receptor for activated C kinase 1) protein stands as a candidate molecular marker for the veterinary diagnosis of malignant melanocytic tumors in horses.⁵²

Recent studies in Spanish Purebred horses and derived breeds such as the Old Kladrubers or the Lipizzaners indicate that there is a genetic link between melanoma and vitiligo.^{45,53,54} Although varying between breeds, the heritability of vitiligo ($h^2 = 0.20–0.63$) is higher than melanoma ($h^2 = 0.07–0.37$).^{45,53,54} Either melanoma or vitiligo predisposition is known to have complex inheritance patterns with polygenic and pleiotropic effects involved. In this sense, Curik and colleagues⁵³ (2013) demonstrated that these diseases are influenced by few genes of moderate-to-large effects (eg, *STX17* and *ASIP* [agouti signaling protein]), as well as a large number of genes with small additive effects, influenced by genes of moderate-to-large effects. However, the study has been limited to investigate the patterns of inheritance and therefore causal mutations have not been yet determined.

The availability of whole genome sequences within the emerged “genomic era” has afforded the opportunity to develop a next-generation high-density SNP array for the domestic horse in 2017.⁵⁵ Numerous genome studies have been performed in large sample designs on many different traits; however, none of the studies reported novel insights into the underlying molecular processes of melanoma and vitiligo diseases in horses. However, a recent study in the Lipizzaner horse identified a common long overlapping homozygous region on ECA14:34.05–35.18 Mb (EquCab2.0), which contains several genes involved in melanoma metastasis and survival rate of melanoma patients in humans (eg, *SPRY4* [sprouty RTK signaling antagonist 4] and *HSP90AB1* [heat shock protein 90 alpha family class B member 1]).⁵⁶ Overall, this approach allowed the identification of potential novel genomic regions that may play a role in

equine melanoma. Further studies including larger cohorts and careful clinical classification of cases are required to better understand the complex genetic and molecular mechanisms leading to skin diseases.

Ehlers-Danlos Syndrome Subtypes: Hereditary Equine Regional Dermal Asthenia and Warmblood Fragile Foal Syndrome

Ehlers-Danlos syndrome (EDS) comprises a group of genetically heterogeneous connective tissue disorders linked with genetic defects affecting collagen or other extracellular matrix proteins.⁵⁷ In horses, EDS subtypes include different inherited connective tissue disorders clinically characterized by skin fragility and hyperextensibility.⁵⁸ To date, 2 EDS subtypes have been described based on causative genetic mutations, hereditary equine regional dermal asthenia (HERDA),^{59,60} and Warmblood fragile foal syndrome (WFFS).^{61,62}

Hereditary Equine Regional Dermal Asthenia

HERDA, also known as hyperelastosis cutis, is a subtype of EDS disorder classified as degenerative skin disease with an autosomal recessive inheritance predominantly observed in the American Quarter Horses.⁵⁹ The defect is in the collagen fibers in the skin and leads to a separation between the epidermis and dermis. The symptoms often start when the horse is broken in to a saddle at around 2 years of age. The pressure from the saddle causes the skin to tear and can lead to wounds with prolonged healing time, which may develop into disfiguring scars. The skin is loose and hyperelastic in affected horses.⁶³

The disease has a prevalence of 3.5% within the general Quarter Horse population, but it is much more prevalent within the cutting horse industry (up to 28%) or within pleasure/working-cow horses (carrier frequency rates of 12.8% and 11.5%, respectively).⁶⁴ A whole-genome scan approach was used to identify a homozygous missense mutation in exon 1 of *PPIB* (peptidylprolyl isomerase B) gene (c.115G > A) in affected HERDA horses.⁵⁹ The gene acts as a chaperon involved in proper folding of collagens, and the mutation affects collagen folding and secretion by a decrease in hydroxylysine and glucosyl-galactosylhydroxylysine in affected horse fibroblasts.⁶⁰ However, not all horses displaying an EDS phenotype have a mutation in the *PPIB* gene, suggesting genetic heterogeneity of EDS disorders.^{65,66}

Warmblood Fragile Foal Syndrome Type 1

WFFS is another subtype of EDS disorder of autosomal recessive inheritance characterized as a fatal defect of the connective tissue involving severe skin malformations in neonatal foals, predominantly observed in Warmblood horses and related breeds.^{61,62} The disease has a carrier frequency in normal adult Warmblood population around ~11%.^{62,67} WFFS is caused by a mutation in the *PLOD1* (procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1) gene (c.2032G > A), which codes for an enzyme important for collagen biosynthesis.⁶²

In summary, 2 different genetic tests are available for the diagnosis of HERDA and WFFS EDS subtypes (Table 2). However, a new case of EDS observed in a Mangalarga-Campolina crossbreed mare with *PPIB* and *PLOD1* mutations tested negative, indicating that another gene involved in the collagen biosynthesis may be responsible for other EDS subtypes.⁵⁸

Chronic Progressive Lymphedema

Chronic progressive lymphedema (CPL) is a disabling skin disorder in draft horse breeds such as Clydesdales, Shire, and Belgian draft horses.^{68,69} It starts at an early

Disease	Breeds	Mode of Inheritance	DNA Test Available
Insect bite hypersensitivity	Multiple breeds (see Table 1). Higher prevalence in Icelandic horse	Complex	No
Melanoma and vitiligo	Gray horses. Higher prevalence in Iberian horses and related breeds	Complex	No
Hereditary equine regional dermal asthenia	Quarter horse and related breeds	Recessive	Yes
Warmblood fragile foal syndrome type 1	Warmblood horses and related breeds	Recessive	Yes
Chronic progressive lymphedema	Draft breeds	Complex	No
Junctional epidermolysis bullosa	Draft breeds, American Saddlebreds	Recessive	Yes
Incontinentia pigmenti	Quarter horse	X-linked dominant	Yes
Hypotrichosis	American Bashkir Curly horse, Missouri Fox trotter, Percheron draft horse	Complex	Yes

age and leads to progressive swelling of the legs and development of severe chronic skin changes such as hyperkeratosis and dermal fibrosis with thick skin folds and nodules. Secondary infections contribute to the chronic changes, and the condition gives rise to severe discomfort to the horse and commonly leads to euthanasia. There seems to be a genetic predisposition to altered elastin metabolism and impaired function of the lymphatic system in the extremities.⁶⁹ The diagnosis is made by clinical picture and by ruling out primary causes to the skin changes such as *Chorioptes*.

CPL has been classified as a multifactorial disorder with a prevalence of 96% within Belgian and some German breeds.⁷⁰ Heritability coefficients for the occurrence of clinical CPL lesions in Belgian Draught Horses have been estimated to be 0.26 for horses older than 3 years.⁷¹ Genetic components are therefore likely to play a role in this disease. However, none of the potential candidate genes known to affect lymphedema-distichiasis syndrome and Darier-White disease (Keratosis follicularis) in humans (eg, *FOXC2* [Forkhead box C2] and *ATP2A2* [ATPase sarcoplasmic/endoplasmic reticulum Ca²⁺ transporting 2]) are associated to CPL in horses.^{72,73}

A whole-genome scan performed across several draft horse breeds affected by CPL identified significant quantitative trait loci on ECA1, 10, and 17.⁷⁴ The study proposed several potential candidate genes involved in the regulation of inflammatory autoimmune responses (eg, *UBE3A* [ubiquitin protein ligase E3A], *CD109* [CD109 molecule], and *MTMR6* [myotubularin-related protein 6]), which will require further functional analysis to confirm whether any of these genes are truly associated with clinical signs observed with CPL. Another GWAS for CPL in Friesian horses did not achieve any genome-wide significance.⁷⁵ However, recent findings in the Belgian Draught horse identified several regions surpassing nominal significance with candidate genes described in previous studies on CPL (eg, *FOXC2*, *UBE3A*, or *CD109*). The study confirms the involvement of several processes of the immune response, thereby supporting the hypothesis to consider CPL as an inflammatory autoimmune response.⁷⁶ Further functional research is needed to identify the genetic cause underlying CPL and the involvement of the immune response.

Junctional Epidermolysis Bullosa

Junctional epidermolysis bullosa (JEB) is a severe skin blistering disease of genetic origin that affects newborn foals. It has an autosomal recessive mode of inheritance and has been found in the Belgian, Italian, and French draft horses and in American Saddlebred horses (see [Table 2](#)).^{77–84} It is a mechanobullous disease that leads to the development of ulcers and blisters in the skin, most commonly at pressure points including the hocks or the stifle and at mucocutaneous junctions such as the mouth and anus. The defect leads to a separation between the skin layers, and only minor trauma leads to severe detachment of the skin and even sloughing of the hoof wall. The foals can also have oral abnormalities with premature eruption of the teeth and loss of enamel that cause bleeding in the mouth. This disease was first called epitheliogenesis imperfecta, but when it was shown that it involved a defect in the lamina propria, it was renamed as JEB. The pathologic signs of JEB closely match what can be observed in Herlitz junctional epidermolysis bullosa in humans, although in horses, the dense hair may act as a protection against trauma.⁷⁹ There is no treatment of JEB, and the foals are euthanized because of animal welfare reasons. The diagnosis can be made from biopsy of intact blisters (macroscopic, histologic, and ultrastructural) or genetic testing in draft and American Saddlebred horses.

Two mutations that affect the dermal-epidermal junction have been identified in horses: one large deletion in *LAMA3* (laminin subunit alpha 3) in American Saddlebred horses and an insertion (1368insC) in *LAMC2* (laminin subunit gamma 2) in Belgians and other draft horse breeds.^{79,80,84} The deletion in American Saddlebred horses is 6589 base pairs and span exons 24 to 27 in *LAMA3*. The mutations affect the anchoring fibril laminin 5 protein located in the basement membrane in the dermal-epidermal junction. Laminin 5 is a heterotrimeric basement membrane protein that consists of 3 glycoprotein subunits—the $\alpha 3$, $\beta 3$, and $\gamma 2$ chains—that are encoded by the *LAMA3* (laminin subunit alpha 3), *LAMB3* (laminin subunit beta 3), and *LAMC2* (laminin subunit gamma 2) genes, respectively.⁸⁴ Identification of healthy carriers is important to prevent the spread of this disease.

Incontinentia Pigmenti

Incontinentia pigmenti (IP) is a congenital skin disorder characterized by abnormalities in the skin but also other structures of ectodermal origin as the teeth and eyes. Based on a single pedigree consisting of 23 horses, the disorder follows an X-linked dominant inheritance pattern, where affected males are aborted and only affected females survive (see [Table 2](#)). The foals develop exudative and pruritic skin lesions soon after birth. These develop to wartlike lesions, and healing is seen as alopecia or occasional wooly hair regrowth. Additional symptoms include dental, hoof, and ocular developmental anomalies.⁸⁵ Horses with IP in this pedigree display many similarities to human IP.⁸⁵

Human familial IP also segregates as an X-linked dominant disorder and is usually lethal prenatally in males. The condition predominantly affects the skin. Cells expressing the mutated X chromosome are eliminated selectively around the time of birth, so females with IP exhibit extremely skewed X-inactivation.⁸⁶ Infants usually have a blistering rash that heals and develops into wartlike skin growths. Hyperpigmentation in a swirled pattern occur in early childhood. In humans, dental, eye, hair, and nail abnormalities can occur.⁸⁶

In human, IP is caused by a mutation in the *IKBK* (inhibitor of nuclear factor kappa B [NF κ B] kinase subunit gamma) gene. The gene is found on the X chromosome (Xq28) and codes for the protein NF κ B's essential modulator (IKK-gamma, formerly called NEMO) found in almost all cells of the body. The function of the IKK-gamma

protein is to activate the protein NFκB inside the cell. NFκB then migrates into the cell nucleus and in turn activates genes that are important for, among other things, fetal development and the function of the immune system.

The IKK-gamma protein is needed for the organism to develop properly, control cell growth and death, and protect cells from infection. This is important, for example, in the early fetal development of ectodermal tissues such as skin, nails, and hair, as well as parts of the central nervous system and the immune system. The most common mutation that causes IP implies a complete loss of function of the protein.

In horses, a nonsense mutation (c.184C > T, p.Arg62*) in *IKBKG* has been identified in mares with IP.⁸⁵ This was achieved by whole-genome resequencing (WGS) of one affected mare and comparison with WGS data from 44 control horses from 11 breeds. The variant is predicted to result in a premature stop codon and truncates approximately 85% of the protein.⁸⁵ Since a homologous mutation (p.Arg62*) has been observed in a human IP patient, horses with the same variant can serve as an animal model for the human condition.

Hypotrichosis

Hypotrichosis implies a less than normal amount of hair. The condition results in alopecia that is apparent at birth or develops during the neonatal period and can be associated with defects in other ectodermal structures such as teeth, hooves, eyes, or sweat glands. It is a genetic disease seen predominantly in the curly coated breeds American Bashkir Curly horses and Missouri Fox trotters. The breeds have normal shedding periods, but, in some individuals, there are permanent hair loss of the mane and tail. It is seen as diffuse to complete alopecia due to dysplasia of hair follicles. Horses with incomplete hypotrichosis are presented with broken hairs and shedding of hairs at the lateral upper tail and at the forelock.

Congenital progressive hypotrichosis has been reported in a blue roan Percheron draught horse.⁸⁷ This horse was born with patchy alopecia of the trunk and legs and at 5 years of age the body hair was almost absent (mane and tail hairs were present, but sparse). There were no abnormalities of the teeth and hoof. Histopathology revealed follicular hypoplasia and hyperkeratosis, as well as an excess of catagen and telogen hair follicles.

Curly coated horse breeds present an exceptional coat that vary in curliness of the body, mane, tail, and ear hairs. Both autosomal recessive and dominant inheritance patterns have been suggested to explain the diversity of the curly phenotype.^{88,89} A GWAS study of 70 curly and straight-haired North American and French horses hypothesized to carry the dominant curly hair trait has been performed.⁹⁰ By using WGS, the study identified a missense variant (g.21891160G > A, p.R89H) in the coil 1A domain of the *KRT25* (keratin 25) gene to be associated with the dominant curly phenotype. The variant was confirmed by genotyping additional 150 curly and 203 randomly chosen straight-haired animals from 35 different breeds. Five discordant curly horses were observed, and sequencing 2 of those horses suggests locus heterogeneity for the dominant curly phenotype within North American Curly horses.⁹⁰

Hypotrichosis is not observed in all curly coated horses. Therefore, a research team aimed at investigating the genetic component of curly coat with and without hypotrichosis.⁹¹ A GWAS analysis identified significant signals on chromosome 11. WGS data detected 2 variants in the region within *KRT25* and *SP6* (transcription factor Sp6) that could explain all hair phenotypes. Curly coat with no hypotrichosis was present in horses carrying only the *SP6* variant. However, a variant allele of the *KRT25* gene was found to be *epistatic* to *SP6*, where horses carrying the *KRT25* variant

(heterozygous or homozygous state), regardless of their *SP6* genotype, exhibited a curly hair phenotype with incomplete or complete hypotrichosis, respectively.

Future Directions

Understanding the molecular basis of mammalian phenotypic traits is a fundamental biological goal and is primarily important for understanding the development of diseases. Equine skin diseases are common, and the interest in equine dermatology is therefore increasing.⁹² For instance, 80% of gray coat horses will develop melanoma after 15 years of age, and up to 60% of horses are susceptible to IBH. Often, there is a genetic component involved in skin disease development, as presented in this article. Notably for monogenic traits where the causative mutation has been identified, genetic testing can serve as a complementing diagnostic tool and could also, when relevant, guide early treatment. However, in domestic animals, genetic testing is of particular value for informed breeding decisions. For instance, genetic testing of a monogenic trait enables identification of healthy carriers of recessive disease alleles (eg, the EDS subtypes HERDA or WFFS).

For complex traits, genomic selection is a more suitable approach. Genomic selection, a form of marker-assisted selection in which genetic markers covering the entire genome are used, is rapidly increasing in importance in other species.^{61,72} The basic concept of genomic selection is to combine whole-genome molecular markers with phenotypic and pedigree data in an attempt to increase the accuracy of prediction for *breeding values*. Genomic selection can be especially valuable for traits that are difficult to measure in large scale, and for lower-heritability traits, such as IBH.

Genomic selection using only significantly disease-associated markers or all markers could increase the efficiency of breeding for decreased IBH disease prevalence.³⁶ This would be of particular value because it can be applied at a young age of the horse, before any mating occurs, as well as because the occurrence of IBH in individual horses highly depends on the exposure to *Culicoides spp.*

Disease mutations may reside either within the coding part of a gene or in a unit that regulates the disease-associated gene. The regulation of gene activity may disrupt protein production and cell processes and result in disease. Therefore, it is important to link variations in the expression of certain genes to the development of each genetic skin disease. A continued focus is to develop and apply genome-based strategies for the early detection, diagnosis, and treatment of equine skin disease. Breeding against genetic disease is an efficient and cost-effective strategy for the management of the disease severity levels, with particular interest in complex traits such as IBH, CPL, or melanoma.

DISCLOSURE

The authors have nothing to disclose.

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