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(54) Title: IDENTIFICATION OF THE CAUSATIVE MUTATION FOR INHERITED CONNECTIVE TISSUE DISORDERS IN EQUINES

(57) Abstract: Provided is a description of a mutation which is positively correlated with Warmblood Fragile Foal Syndrome Type 1 (WFFST1). The mutation is a G to A change at a specific location in the equine lysyl hydroxylase 1 (LH1) gene. Compositions and methods for use in diagnosing WFFST1 are provided.

IDENTIFICATION OF THE CAUSATIVE MUTATION FOR INHERITED CONNECTIVE TISSUE DISORDERS IN EQUINES

This application claims priority to U.S. patent application serial no. 61/486,464, filed on May 16, 2011, the disclosure of which is incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates generally to inherited disease observed in horses. More particularly, the invention relates to detecting a point mutation in a gene associated with Fragile Foal Syndrome Type 1.

DESCRIPTION OF RELATED ART

Inherited connective tissue disorders occur in a variety of species. They are known by many names and are associated with many different underlying genetic defects. Nonetheless, they all present with skin hyperextensibility. Warmblood Fragile Foal Syndrome Type 1 (WFFST1) occurs in the Warmblood horse population which are a group of mid-sized horse types primarily originating in Europe, developed with the aim of competing in Olympic equestrian sports (often called Sport Horses). The presently available pedigree data shows that Warmblood Fragile Foal Syndrome Type 1 segregates in the Hanoverian, Selle Francais, KWPN (Dutch), Oldenburg, and Westphalian lines. These registries, as well as the Holsteiner, Wurtemberger, Rhinelander, Gelderlander, Zweibrucker and Bavarian Warmblood are central to all Warmblood/Sport Horse registries, placing all horses among the broader group of registries at risk for carrying this disorder. A partial list of many of the registries includes: Hanoverian; Selle Francais; KWPN; Oldenburg; Rhinelander; Holsteiner; Westphalian; Gelderlander; American Warmblood; Anglo-Norman; Austrian Warmblood; Bavarian Warmblood; Belgian Warmblood (including Belgian Half-blood); Canadian Sport Horse; Danish Warmblood; Brazilian Sport Horse; Friesian Sport Horse; German Warmblood ZfDP; Czech Warmblood; Irish Sport Horse; Karossier/Ostfriesen/Alt-Oldenburger; Hungarian Warmblood; Romanian Sport HorseSwedish Warmblood; Swiss Warmblood; Wurttemberger; and Zweibrucker. There is an ongoing an unmet need to identify the mutation that causes WFFST1 and to develop

compositions and methods for determining which horses carry it and which horses do not. The present invention meets these and other needs.

SUMMARY OF THE INVENTION

The present invention is based on the discovery of the mutation associated with WFFSTl. The method comprises testing a biological sample obtained or derived from a horse to identify the presence of a G to A mutation in the equine lysyl hydroxylase 1 (LHl) gene. The location of the change will be apparent to those skilled in the art from the amino acid and nucleotide sequences described herein. In one embodiment, the mutation is at a nucleotide position 2086 of the cDNA produced using mRNA transcribed from an LHl gene which contains the mutation. This cDNA is presented in Figure 4 as SEQ ID NO:4. Determining a G to A mutation in only one allele establishes that the horse is heterozygous for the WFFSTl mutation and can thus be identified as a carrier. Determining the G to A mutation in both alleles establishes that the horse is homozygous for the WFFSTl mutation and can thus be identified as affected or predisposed to developing WFFSTl. Determining an absence of the G to A mutation in both alleles establishes that the horse is homozygous for the absence of the allele and is indicative that the horse is genetically normal with respect to WFFSTl.

The mutation can be determined from DNA or RNA, or from a cDNA amplified from RNA, or from protein obtained from a horse. In one embodiment, the presence of the mutation is identified by determining the presence of a polynucleotide comprising the sequence of SEQ ID NO: 1, which is a PCR product obtained by amplification of a segment of the LHl gene from a horse, the genome of which comprised the mutation. In another embodiment, determining the presence of the mutation comprises determining a change from G to A at position 4 in the sequence of SEQ ID NO: 6, which is the wild type sequence of exon 19 of the equine LHl gene. The sequence of exon 19 comprising the mutation is presented as SEQ ID NO:7.

In various embodiments, determining the presence of the WFFSTl mutation (irrespective of heterozygosity or homozygosity for it) establishes that the horse is not genetically normal with respect to WFFSTl. Determining the absence of the mutation

(irrespective of heterozygosity or homozygosity for its absence) establishes that the horse is not affected with WFFST1.

Also provided is a method for selecting horses for breeding. This aspect of the invention comprises obtaining a biological sample from a horse, testing the biological sample to detect the WFFST1 mutation and, based on analysis of the allelic status for the WFFST1 mutation, breeding horses that are genetically normal or heterozygous for it with horses that are genetically normal with respect to WFFST1.

Isolated nucleic acids and proteins which comprise the mutation are also provided by the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A provides a photographic representation of excessive skin elasticity that is typical of horses affected with WFFST1.

Figure IB provides a photographic representation of a skin lesion that is typical of horses affected with WFFST1.

Figure 2 provides the sequence of wild type equine LH1 cDNA (SEQ ID NO:2). The wild type bases at the positions of the mutation (base 2086) is bold and enlarged. Non-informative non-coding region single nucleotide polymorphisms (SNPs) are located at bases 2320 and 2580 (when the A of the ATG translational start codon beginning at position 55 is designated as nucleotide 1). Either G or A may occur at these positions in wild-type alleles. Thus there are two additional nucleotides depicted in this sequence in Figure 2 than would exist in the cDNA, which is for convenience of illustrating this variability. In the depicted sequence the A of the ATG codon is at position 55. The translational stop codon is position 2182-2184 when designating the A at position 55 as nucleotide number 1.

Figure 3 provides the wild type equine LH1 polypeptide sequence (727 amino acids; SEQ ID NO:3). The position of the amino acid in the normal peptide corresponding to the position of the change in mutation is bold and enlarged.

Figure 4 provides the sequence of a mutant equine LH1 cDNA (SEQ ID NO:4). The position of the mutation is 2032 (when the A at position 55 is designated nucleotide 1), and non-informative, non-coding region SNPs are bold and enlarged. These are respectively at 2320 and 2580. The A of the ATG codon at 55 is bold and enlarged. The translational stop codon is position 2182-2184, and is bold and enlarged. When the first nucleotide of the

sequence is counted as nucleotide number 1, the mutation is at position 2086 and the other features of the sequence described above can take their numerical positions in relation to this numbering convention.

Figure 5 provides the amino acid sequence of the mutant equine LH1 peptide (SEQ ID NO:5). Arginine replaces the evolutionarily conserved Glycine at position 678 (R is bold and enlarged).

Figure 6 provides the sequence of the wild type equine LH1 exon 19 (SEQ ID NO:6). The wild type base at the position of the mutation (base 4) is bold and enlarged and non-coding region SNPs are also shown in bold and enlarged. These are respectively at bases 292 and 552, and either G or an A may occur at these positions in wild-type alleles, while an A occurs at each of these positions in the WFF1 mutant allele. The translational stop codon is bold and enlarged.

Figure 7 provides the sequence of the mutated equine LH1 exon 19 (SEQ ID NO:7). The mutation (base 4) is shown in bold and enlarged. The non-informative non-coding region SNPs are also bold and enlarged and occur at bases 292 and 552. The translational stop codon is shown in bold and is enlarged.

Figure 8 provides the sequences of exons 1-18 of the equine LH1 gene (SEQ ID NOs 8-25, respectively).

Figure 9A provides the nucleotide sequence of a representative amplicon obtained by PCR amplification of an equine LH1 gene that does not contain the mutation (SEQ ID NO:26).

Figure 9B provides the nucleotide sequence of a representative amplicon obtained by PCR amplification of an equine LH1 gene that does contain the mutation. The mutation is at position 147 and is enlarged and shown in bold (SEQ ID NO:l).

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based on the discovery of a mutation which is positively correlated with WFFSTl. The mutation is a G to A change in the equine LH1 gene. The mutation is referred to herein as the "WFFSTl" mutation. The invention comprises a method for analysis of WFFSTl status in a horse. The method comprises obtaining a biological sample from a horse and testing nucleic acids and/or proteins from the biological sample to determine the presence or absence of the WFFSTl mutation. Determining a G to

A mutation in only one allele establishes that the horse is heterozygous for the WFFSTl mutation. The horse can thus be identified as a carrier of WFFSTl. Determining the G to A mutation in both alleles establishes that the horse is homozygous for the WFFSTl mutation. The horse can thus be identified as affected or predisposed to developing WFFSTl. Determining an absence of the G to A mutation in both alleles establishes that the horse is homozygous for the absence. The horse can accordingly be identified as genetically normal with respect to WFFSTl. An LH1 allele that does not comprise the WFFSTl mutation and is thus genetically normal with respect to WFFSTl is also referred to herein as "wild type." In one embodiment of the invention, determining the presence of a wild type WFFSTl allele establishes that the horse is not affected or predisposed to developing WFFSTl. In another embodiment, determining the presence of a WFFSTl mutation establishes that the horse is not genetically normal with respect to WFFSTl.

The location of the G to A change in any particular sample of equine nucleic acids can be ascertained by the skilled artisan via reference to the nucleotide sequences presented herein. The invention encompasses detecting the presence or absence of the WFFSTl mutation in any polynucleotide from any suitable biological sample obtained or derived from a horse. Detecting the mutations by testing the polypeptide encoded by the LH1 gene is also included. Further, for each DNA sequence described herein its RNA equivalent (replacing each T with a U) is included in the invention. All polynucleotides which encode the equine LH1 polypeptides presented herein are also included with the scope of the invention.

The WFFSTl mutation is shown in one illustrative embodiment as an A at position 147 of SEQ ID NO: 1 (Fig. 9B). SEQ ID NO: 1 is the sequence of an amplicon obtained by PCR amplification of a sample comprising nucleic acids which contained the mutation. The A at position 147 of SEQ ID NO: 1 represents a change from a wild type G to the mutated sequence which comprises an A at position 147. The sequence of an amplicon obtained by PCR amplification of a sample which did not contain the mutation in presented in SEQ ID NO:26 (Fig. 9B).

Other polynucleotide sequences presented herein show the mutation in extended equine genetic sequences. For example, the WFFSTl mutation is also shown as an A at position 2086 in the sequence of SEQ ID NO:4. This WFFSTl mutation can be designated to be at position 2032 of SEQ ID NO:4 if nucleotide numbering in that sequence begins

with the A at position 55, which is the A of the ATG translation initiation codon. SEQ ID NO:4 is the sequence of a cDNA produced from mRNA transcribed from an LHl gene which contains the mutation. Non-informative, non-coding region SNPs are present at positions 2320 and 2580 (numbering of nucleotides as if the A of the ATG translational start codon is designated as nucleotide position 1). The non-informative SNPs can be A or G at these positions. The pertinent translational stop codon is position 2182-2184 (again where numbering using A at position 55 of the cDNA is designated as nucleotide position 1).

In another embodiment, determining the presence of the mutation comprises determining a change from G to A at position 4 in the sequence of SEQ ID NO:6. In connection with this, the wild type sequence of exon 19 of the equine LHl gene as shown in SEQ ID NO:4 comprises G at position 4. The sequence of exon 19 comprising the WFFST1 mutation is provided in SEQ ID NO: 7. Thus, the invention includes determining the presence of the mutation by determining the sequence of SEQ ID NO:7 from a sample obtained from a horse, or determining the absence of the mutation by determining the sequence of SEQ ID NO:6 from a sample obtained from a horse.

Those skilled in the art will recognize from the foregoing description that position 2086 in SEQ ID NO:2, position 2086 in SEQ ID NO:4, position 147 in SEQ ID NO:l, position 147 in SEQ ID NO:26, position 4 in SEQ ID NO:6, and position 4 in SEQ ID NO:7 are equivalent expressions of the location of the site where the G to A change that constitutes the WFFST1 mutation takes place at the genomic DNA level.

Those skilled in the art will also recognize that probes and primers can be developed for detecting LHl sequences that contain the mutation or for LHl sequences that do not contain the mutation based on the nucleotide sequences described herein. For example, any fragment of SEQ ID NO:2 or SEQ ID NO:4, or their complements, that is at least 12 nucleotides long and does not encompass the mutation site can be used as a primer for any conventional amplification or sequencing reaction to perform the method of the invention. Any fragment that is at least 12 nucleotides long and which does encompass the mutation site can be used for detecting the presence or absence of the mutation using any conventional technique that involves hybridization of a diagnostic probe that can discriminate between an A or a G at the mutation site. In specific embodiments, PCR can be performed on DNA obtained from a horse with a first primer that is identical or complementary to nucleotides 1-24 of SEQ ID NO: 1 and a second primer which is identical

or complementary to the 25 nucleotides at the 3' end of SEQ ID NO: 1. Those skilled in the art will recognize that the sense orientation of the first and second primers will be opposite according to conventional PCR methods.

The WFFST1 mutation results in a change in the amino acid sequence encoded by the LHl gene which comprises it. In particular, an equine LHl polypeptide sequence encoded by an equine LHl gene which contains the mutation is presented in SEQ ID NO:5. This polypeptide differs from the wild type sequence (a polypeptide sequence encoded by an equine LHl gene that does not contain the mutation, depicted in SEQ ID NO:3) at amino acid position 678. In the mutant sequence the amino acid at position 678 is Arginine, while in the wild type sequence this amino acid position is occupied by Glycine. Thus, in various embodiments, the invention provides for testing LHl protein from a biological sample obtained or derived from a horse to determine the amino acid at position 678. In one embodiment, detecting LHl protein comprising Arginine at position 678 establishes that the horse that produced it is not genetically normal with respect to the WFFST1 mutation.

The invention can be carried out using any suitable biological sample obtained from a horse. In various embodiments, the biological sample contains nucleic acids encoding the LHl protein or portions thereof, or a biological sample that contains the LHl protein, or combinations of such polynucleotides and protein. Suitable sources of biological sample include but are not limited to blood, hair, mucosal scrapings, semen, tissue biopsy, or saliva. In one embodiment, the biological sample is blood. In one embodiment, the biological sample is obtained from the horse and used directly in analyzing the polynucleotides and/or protein. In another embodiment, the biological sample is obtained from the horse and subjected to a processing step before the analysis. In some examples, the processing step can be carried out to isolate or purify the polynucleotides and/or proteins to be analyzed, or to amplify the polynucleotides. Thus, the invention in certain embodiments comprises extracting a biological sample from a horse so that the sample is separated from its naturally occurring environment, and mixing it with a non-naturally occurring reagent, such as an extraction buffer. The sample can be mixed with organic solvents, such as formaldehyde, chloroform, alcohols, and the like. The sample may also be subjected to successive heating and cooling steps, or freezing temperatures, such as -20 Celsius.

While any suitable technique can be used to detect the presence or absence of the WFFST1 mutation, presently preferred techniques include polymerase-chain based

reactions, such as PCR or real-time PCR (RT-PCR), or reverse-transcriptase based PCR, or restriction digestions. These processes involve well known techniques and generally include providing a composition comprising isolated nucleic acids for testing and mixing the nucleic acids with non-naturally occurring reagents, such as recombinant or isolated bacterial, viral or bacteriophage polymerases, buffers comprising pre-fixed concentrations of free deoxyribonucleotides, including those that are unlabeled and/or those that are detectably labeled, and/or recombinant bacterial restriction endonucleases, and combinations of the foregoing. The compositions can comprise one or more synthetic oligonucleotides that have complete or partial complementarity to the isolated equine polynucleotide so that they can function as probes, such as primers. In another embodiment, an isolated polynucleotide which comprises or might comprise the WFFST1 mutation can be annealed to a synthetic oligonucleotide that is fixed to a substrate, such as glass or a resin. For example, an isolated polynucleotide which comprises or is suspected of comprising the WFFST1 mutation can be annealed to a synthetic oligonucleotide that is present on an array which comprises a plurality of distinct oligonucleotides. Such arrays can distinguish polynucleotides that differ from one another by a single nucleotide and will produce a signal that is detectable by an individual and/or a machine to signify the presence or the absence of the mutation.

In another embodiment, the presence or the absence of the WFFST1 mutation in a polynucleotide can be determined using a restriction endonuclease digestion. For example, when present the WFFST1 mutation introduces a unique AlwNl site. Using a polynucleotide consisting of SEQ ID NO: 1 hybridized to its complement as a non-limiting example, digesting this amplicon with AlwNl yields fragments of -150 and 200 bp on digestion, as compared to the uncut 350 bp fragment obtained when the wild-type allele is present. Furthermore, the WFFST1 mutation ablates an Acil site, yielding fragments of -269, 10 and 72 bp, while digestion of the wild-type fragment produces -149, 120, 10 and 72 bp bands in this illustrative exmaple. Thus, digestion with Acil cleaves the wild-type product in three places while the mutant product is only cleaved in two places. Those skilled in the art will recognize that there are a multitude of restriction endonuclease assays that can be developed if desired from these observations.

As with polynucleotides, detecting a protein which comprises the WFFST1 mutation can be performed using any known technique. Such techniques include but are not limited

to immunodetction assays, which entail forming a complex between a protein that comprises or might comprise the amino acid change encoded by the WFFSTl mutation with a monoclonal antibody that is produced by a hybridoma or by recombinant methods. The complex can include a detectably labeled ligand, such as a detectably labeled monoclonal antibody that is specific for the protein. The invention includes generation and use of monoclonal antibodies that can discriminate between the wild type and mutant forms of the LSI protein.

Also provided in the present invention are kits for detecting the presence or absence of the WFFSTl mutation. The kits comprise reagents for nucleic acid based detection of the presence or absence of the mutation(s), or antibodies for detecting the presence or absence of the mutation. In one embodiment, the kits comprise reagents for extraction/preparation of nucleic acid samples and pair(s) of specific primers for use in identification of the mutation. In another embodiment, the kits provide antibodies and compositions used for probing samples with the antibodies to determine whether or not the WFFSTl mutation is present in a horse from which a biological sample is obtained and tested according to the invention. Any of the aforementioned kit components can be provided in one or more sealed, sterile containers, such as vials.

It is contemplated that any horse at any developmental stage (i.e., fetal, neonatal, postnatal, juvenile, adult, etc.) may be tested according to the method of the invention. Further, any horse, regardless of breed, could be homozygous or heterozygous for the WFFSTl mutation, or homozygous for the absence of the mutation, and thus is suitable for testing. In various embodiments, the horse may be a Warmblood, Thoroughbred, Standardbred, Tennessee Walking Horse, Mustang, Quarter Horse, Arabian, Draft breed, Minature Horse, or a pony.

By using the tools and method described herein, horses which are genetically normal for the disease, carriers of the WFFSTl disease and horses which are affected by (or predisposed to) WFFSTl can be identified. Upon identification, affected or predisposed, or carrier horses can be eliminated from the breeding stock. Alternatively, horses which are affected or predisposed with WFFST, or carriers of the WFFST mutation, can be mated with genetically normal horses to avoid producing affected foals.

In one embodiment, the method further comprises fixing the result of determining the presence or absence of the WFFSTl mutation in a tangible medium, such as a paper

report, or a portable medium on which electronic files can be stored, such as a hard drive, a computer disk, a thumb drive, and the like. In another embodiment, the invention includes communicating the result of determining the presence or absence of the WFFSTl mutation to an individual. The test result can be communicated to an individual by any method. Non-limiting examples of the individual to whom the test results may be communicated include the horse owner, an equine registry, a veterinarian, or a provider of genetic test results.

The WFFSTlphenotype is clinically detectable and present at birth (premature birth has been reported). Affected foals are euthanized within days to weeks of life due to the poor prognosis. Horses that are clinically affected can be recognized by skin traits that are characteristic of WFFSTl. Figs 1A and IB illustrate typical manifestations of WFFSTl, where the skin lacks tensile strength (Fig. 1A) and extreme skin fragility characterized by tearing, ulceration, etc. from contact with normal surroundings; Fig IB). Lesions can occur anywhere on the body, but are most noted on pressure points and in addition to skin wounds, lesions are found on the gums and other oral cavity mucous membranes and the perineum. Skin is hyperextensible (stretchy) over essentially the whole body, and especially the head, neck, thorax and limbs. Limb joints are lax and hyperextensible. Fetlocks are the most dramatically affected and affected foals cannot stand normally. Ears may be soft, overly flexible, bent or floppy presumably due to failure of collagen in the supporting cartilage. At post mortem exam hematomas and seromas are noted under the skin, particularly over pressure points. Supporting structures around joints (capsule, tendons, ligaments) are lax. Lesions of articular cartilage are reported in some joints.

The invention will be further understood by the following Example, which is intended to be illustrative and not restrictive in any way.

Example 1

This Example demonstrates a 100% correlation between homozygosity (AA) for the causative mutation and the disease phenotype. The disease phenotype is recognizable on clinical examination and is characterized by the presence at birth of soft skin lacking adequate tensile strength to withstand even normal environmental contact. The skin is hyperextensible (lacking normal recoil when traction is applied/released) over the whole

body, especially the head, neck, thorax and limbs. Over the first hours to days of life, focal seromas, hematomas, and lacerations/ulcerations result from incidental environmental contact. Such lesions are predominantly found over pressure points such as fetlocks (metacarpal-phalangeal joints), carpi (knees), and tarsi (hocks), but may occur anywhere on the body (including oral cavity and perineum). Limb joints, particularly the fetlocks) are lax and hyperextensible, and affected foals cannot typically stand normally. The ears are typically bent or floppy. Also demonstrated is a 100% correlation between heterozygosity (AG) and the normal phenotype, as well as a 100% correlation between homozygosity for wild type (GG) and the normal phenotype. The normal phenotype lacks the clinical criteria specified above for the disease phenotype.

Results from determining genotype and phenotype are presented in Table 1. To obtain the results presented in Table 1, the WFFST1 genotype was established by PCR amplification of a 350 bp fragment of the LH1 gene containing the WFFST1 mutation site from genomic DNA, followed by automated direct sequencing of the resulting PCR products. The sequences of amplified fragments (amplicons) representing the wild-type and WFFST1 alleles are given in SEQ ID NO: 26 and SEQ ID NO: 1, respectively. Genomic DNA was extracted from whole blood/serum or mane/tail hair root samples using respectively, the QIAamp DNA Blood Mini Kit or the DNeasy Blood and Tissue Kit (QIAGEN) according to the manufacturer's recommended protocols. Amplifications were performed in 50 microliters total volume containing 3 microliters genomic DNA (~40 ng), 1 X Platinum Taq Hi Fi buffer, 200 micromolar dNTPs, 0.2 micromolar each primer, and 1 unit Platinum Taq Hi Fi (Invitrogen). Primer sequences for PCR were 5'-atagctgtcactccacaaggcaca-3' (sense primer; SEQ ID NO:27) and 5'-cagtggttgttggcaacgaggaaaa-3 ' (antisense primer; SEQ ID NO:28). DNA amplification was performed in an ABI 2720 thermal cycler using the following program: Initial denaturation: 5 min at 95°, followed by 40 cycles of: 30 sec at 95°; 30 sec at 55°; 30 sec at 72°; followed by a final extension segment of 5 min at 72°. Unincorporated primers and nucleotides were eliminated from PCR reactions using the DNA Clean & Concentrator-5 Kit (Zymo Research) according to the manufacturer's recommended protocol. DNA sequencing was performed by cycle sequencing using a primer having the sequence 5'-atagctgtcactccacaaggcaca-3', using dye terminator chemistry (Big Dye v3.1, Applied Biosystems, Foster City CA), followed by dye terminator removal using Edge Performa

DTR gelfiltration plates (Edge Biosystems, Gaithersburg MD) and automated data collection on an Applied Biosystems 3730x1 DNA Analyzer.

The results presented in Table 1 demonstrate a 100% association between the presence of a G to A mutation in the LHl gene at position 2086 in the sequence of SEQ ID NO:4. More specifically, there is a 100% association between homozygosity (A/A) for the causative mutation and the disease phenotype; a 100% association between heterozygosity (A/G) and the normal phenotype, as well as a 100% association between homozygosity for wild type (G/G) and the normal phenotype.

Table 1

Genotype	Homozygous for the mutant allele (A/A)	Heterozygous for the mutant allele (A/G)	Homozygous for the normal allele (G/G)
Number Tested	2	8	149
Clinical Phenotype	2 Affected	8 Normal	149 Normal

The data summarized in Table 1 show that two foals homozygous for the mutant (A) allele were clinically affected. The phenotype of affected foals was determined by clinical examination over the initial days of life. Eight Warmblood horses were determined to be heterozygous for the mutant allele (A/G) and all eight were clinically normal. Three of the eight heterozygotes were obligate carrier parents of the affected foals. The five additional heterozygotes were identified by screening DNA samples from 104 clinically normal Warmbloods from the local population. One hundred and forty-nine horses were determined to be homozygous for the normal allele (G) and all were clinically normal. This group of horses included 50 horses of various breeds (Thoroughbred, Standardbred, Tennessee Walking Horse, Mustang, Quarter Horse, Arabian, Drafts and ponies) in addition to 99 clinically normal Warmbloods.

The results in Table 1 show clearly that there is a 100% association (2/2 horses) between homozygosity for the causative mutation and the affected phenotype. The frequency of the mutant allele (A) appears to be approximately 3.77% in the test population. The frequency of the mutant allele (A) in the "at-risk" population (Warmbloods) appears to be approximately 5.50%, while the carrier frequency among the group of clinically normal adult Warmbloods studied is 7.48%. Mutations in lysyl hydroxylase 1 (LHl) are known to cause the human disorder Ehlers-Danlos Syndrome VI (EDSVI). This is an autosomal recessive connective tissue fragility syndrome characterized by premature birth, infant floppiness/joint laxity, development of kyphosis and/or scoliosis, skin fragility, abnormal bruisability and scarring, ocular abnormalities, recurrent joint dislocation, cardiac valvular failure, catastrophic arterial and/or bowel rupture. The present invention is believed to represent the first discovery of a spontaneously occurring EDSVI analog in a non-human animal species, and mutations causative of deficient/abnormal LHl are believed to have not previously been described in non-human animals.

The invention has been described through specific embodiments. However, routine modifications to the compositions, methods and devices will be apparent to those skilled in the art and such modifications are intended to be covered within the scope of the invention.

We claim:

1. A method for determining whether a horse is heterozygous, homozygous or has an absence of a Warmblood Fragile Foal Syndrome Type 1 (WFFSTl) mutation, wherein the WFFSTl mutation comprises a change from G to A at position 147 in the sequence of SEQ ID NO:26, the method comprising: obtaining a biological sample from the horse and determining from the biological sample the presence or absence of the WFFSTl mutation, and identifying the horse as WFFSTl mutation homozygous by determining homozygosity for the WFFSTl mutation; or identifying the horse as WFFSTl mutation heterozygous by determining heterozygosity for the WFFSTl mutation; or identifying the horse as genetically normal with respect to WFFSTl by determining a homozygous absence of the WFFSTl mutation.

2. The method of claim 1, wherein the determining the presence or absence of the WFFSTl mutation comprises isolating DNA or RNA from the biological sample and testing the isolated DNA and/or RNA for the presence or absence of the WFFSTl mutation.

3. The method of claim 1, wherein the determining the presence or absence of the WFFSTl mutation comprises processing the biological sample to isolate DNA which comprises or might comprise the WFFSTl mutation, subsequently amplifying a segment of the isolated DNA which comprises or might comprise the WFFSTl mutation to obtain a DNA amplification product, and testing the DNA amplification product to determine the presence or absence of the WFFSTl mutation.

4. The method of claim 3, wherein the determining the presence or absence of the WFFSTl mutation comprises separating RNA from the biological sample, creating a cDNA from the separated RNA, and testing the cDNA to determine the presence or absence of the WFFSTl mutation.

5. The method of claim 1, wherein the determining the presence or absence of the WFFST1 mutation comprises isolating protein from the biological sample and testing the isolated protein for the presence of a protein comprising the sequence of SEQ ID NO:5, wherein the presence of a protein comprising the sequence of SEQ ID NO:5 establishes the presence of the WFFST1 mutation.

6. A method for determining whether a genome of a horse comprises a Warmblood Fragile Foal Syndrome Type 1 (WFFST1) mutation, wherein the WFFST1 mutation comprises a change from G to A at position 4 in the sequence of SEQ ID NO:6, the method comprising: obtaining a biological sample from the horse and testing the biological sample to determine the presence or absence of the WFFST1 mutation.

7. The method of claim 6, wherein the determining the presence of the mutation comprises determining the presence of SEQ ID NO:7.

8. The method of claim 6, wherein the determining the absence of the mutation comprises determining the presence of SEQ ID NO:6.

9. The method of claim 6, comprising determining the absence of the WFFST1 mutation and identifying the horse from which the biological sample was obtained as not affected with WFFST1.

10. The method of claim 6, comprising determining the presence of the WFFST1 mutation and identifying the horse from which the biological sample was obtained as not genetically normal with respect to WFFST1.

11. The method of claim 6, comprising determining homozygosity for the WFFST1 mutation and identifying the horse from which the biological sample was obtained as affected with or predisposed to WFFST1.

12. The method of claim 6, comprising determining heterozygosity for the WFFST1 mutation and identifying the horse from which the biological sample was obtained as a carrier of the WFFST1 mutation.

13. The method of claim 6, wherein the determining the presence of the WFFST1 mutation comprises detecting from the biological sample a polypeptide comprising the sequence ofSEQ ID NO:5.

14. A composition comprising an isolated nucleic acid, wherein the isolated nucleic acid comprises the sequence of SEQ ID NO: 1.

Figure 1A 1/10

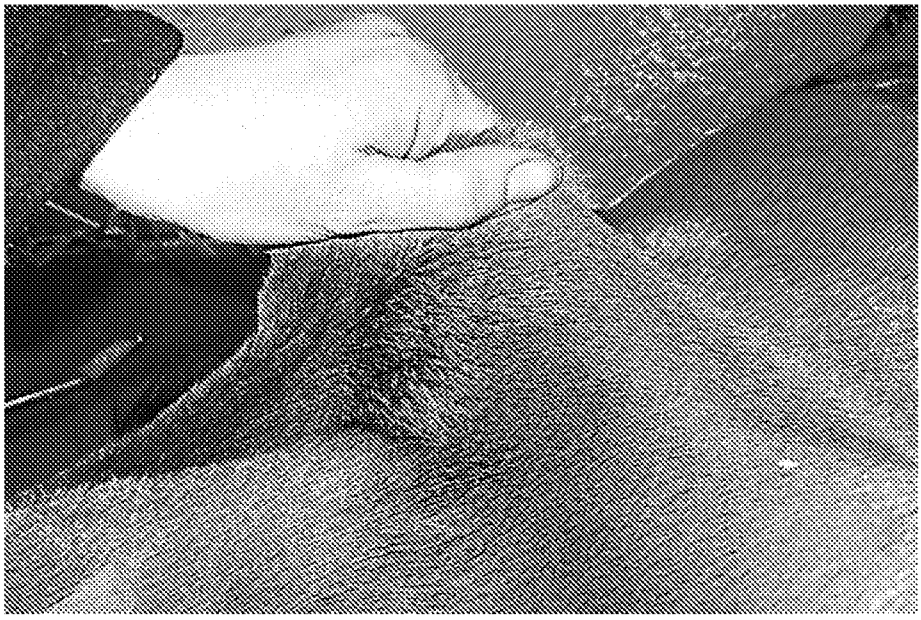

Figure 1B

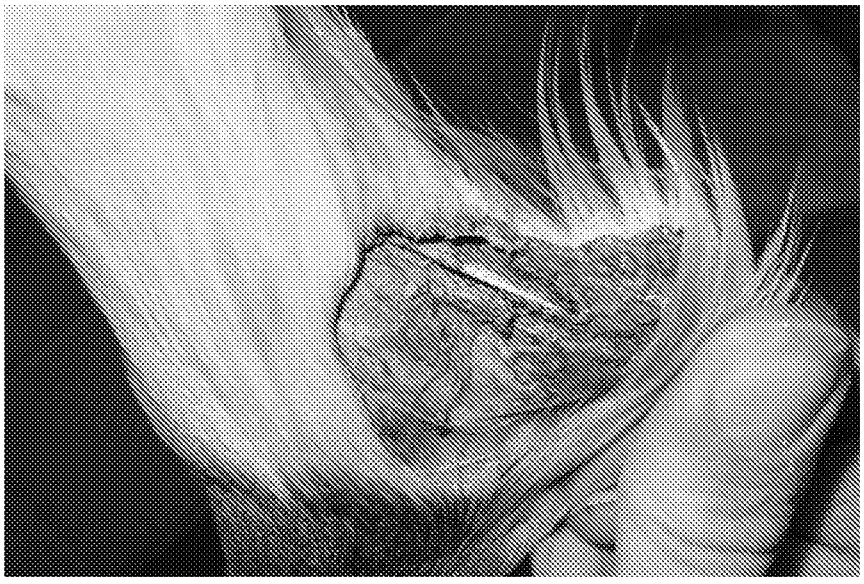

Figure 2 2/10

Wild Type Equine LH1 cDNA (SEQ ID NO:2)

AGTTTCCAGCCTGCCAGCGCCTGCTGGCTGCCCGGACGTCCCCAGACCTCGGCC
ATGCGGCCTCTGCTGCTCCTGGCCCCGCTGGGCTGGCTGCTCCTGGCCGAAG
CGAAGGGCGACGCCAAGCCGGAGGACAACCTCTTAGTCCTCACGGTGGCCACG
AAGGAGTCCGAGGGGTTCCGACGCTTCAAGCGCTCAGCCCAGTTCTTCAACTAC
AAGATCCAGGCGCTGGGCCTGGGGGAGGACTGGGACGGGGACAAGGAGACGT
CAGCGGGCGGCGGGCTGAAGGTTCGGCTGCTGAAGAAAGCTCTGGAGAAGCAT
GCAGACAAAGAGAACCTGGTCATTCTCTTCACAGACAGCTATGATGTGGTATTT
GCCTCGGGGCCCCGAGAGCTCCTGAAGAAGTTCCGGCAGGCCAGGAGCCAGGT
GGTCTTCTCGGCCGAGGAGCTCATCTACCCCGACCGCAGGCTGGAGGCCAAGTA
CCCGGTGGTGTCCGATGGCAAGAGGTTCCTGGGCTCTGGAGGCTTCATCGGTTA
TGCCCCCAACCTCAGCAAACTGGTGGCTGAGTGGGAGGGCCAGGACAGCGACA
GTGACCAGCTGTTTTATACCAAGATCTTCTTGGACCCAGAGAAGAGGGAGCGG
ATCAACATCACCCTGGACCACCGCTGCCGTATCTTCCAGAACCTGGTTGGAGCC
TTAGATGAGGTCGTGCTCAAGTTTGAAATGGGCCATGTGAGGGCGCGGAACCT
GGCCTACGACACCCTCCCCGTCCTGATTCATGGCAACGGGCCCACCAAGCTGCA
GCTGAACTACCTGGGCAACTATATCCCTCGCTTCTGGACCTTCGAGACGGGCTG
CACGGTGTGTGACGAGGGCCTGCGCAGCCTCAAGGGCATTGGGGATGAAGCTC
TGCCTGTGGTCTTGGTCGGCGTGTTCATCGAGCAGCCCACGCCGTTCCTGTCCCT
GTTCTTCCAGCGGCTTCTGCGCCTGCATTACCCCCGGAAACAGCTGCGGCTTTTT
ATTCATAACCATGAGCAGCACCACAAGGCTCAGGTGGAGCAGTTCCTGGCAGA
GCATGGCGGCGAGTACAAGTCTGTGAAACTGGTGGGCCCCGAGGTGCGGGTGG
CAAACGCCGATGCCAGGAACATGGGCGCGGACCTGTGCCGGCAGGACCGTGGC
TGCACCTACTACTTCAGTGTGGATGCCGACGTGGCCCTGACCGAGCCCAAGACC
CTGCGACTGCTGATTGAGCAGAACAAGAATGTCATCGCCCCGTTGATGACCCGC
CACGGGAGGCTGTGGTCGAACTTCTGGGGGGCAATGAGTGCAGATGGCTACTA
CGCCCGCTCCGAGGACTACGTGGACATTGTGCAGGGGCGGCGTGTTGGCGTCTG
GAACGTGCCCTACATCTCGAACATTTACCTGATCAAGGGCAGTGCCCTGCGGGC
TGAGCTGCAGCAGACAGATCTGTTCCACCACAGCAAGCTGGATGCCGACATGG
CCTTCTGTGCCAATATCCGGCAGCAGGATGTGTTCATGTTCCTGACCAACCGGC
ACACCTTCGGCCACCTGCTCTCCCTGGACAGCTACCAGACCACCCACCTCCACA
ACGACCTCTGGGAGGTGTTTAGCAACCCCGAGGACTGGAAGGAGAAGTACATC
CATGAGAACTACACCAGGGCCCTGGCGGGGAAGCTGGTGGAGATGCCTTGCCC
GGATGTCTACTGGTTCCCCATCTTCACGGAGACGGCCTGTGACGAGCTGGTGGA
GGAGATGGAGCACTACGGCCAGTGGTCTCTGGGAGACAATAAGGACAACCGCA
TCCAGGGTGGCTACGAAAATGTGCCGACCATCGACATCCACATGAACCAGATC
AGCTTTGAGCGGGAGTGGCACAAGTTCCTGGTGGAGTACATCGCCCCCATGACA
GAGAAGCTGTATCCAGGCTACTACACCAGGGCCCAGTTCGACCTGGCCTTTGTT
GTCCGCTACAAGCCTGACGAGCAGCCCTCGCTGATGCCCCACCACGATGCCTCC
ACCTTCACTGTCAACATCGCCCTGAACCGGGTCGGGGTGGATTACGAGGGC**G**G
GGGCTGTCGGTTCCTGCGTTACAACTGCTCCATCCGAGCCCCACGGAAGGGCTG
GACCCTCATGCACCCCGGACGACTCACGCACTACCACGAGGGACTCCCCACCAC

Figure 2, continued

CAAGGGCACCCGCTACATCGCGGTCTCCTTCGTCGATCCC**TAA**TCGGCCAAGC
CTGGCCACTTCGGACCTTTTCCTCGTTGCCAACAACCACTGCCCAGCAGCCTCTG
GGGCCTTGGGGTCCCAGGGAACCTGGTCCAGCCTCCGGGCTCTTGACCTCCCAT
TGCTTTCGGAGCCGCC**G/A**TCGGAGAGACTGGGCCGCAGGCCAGAGGCAGAGC
ACACCTCCTTGGCTGGGGCTTTCCTGGTGTTCTGCTCCCCACCCCGGGAGATGG
GGTCCACGCTCACTGCCTTGTAACAGCTCATCCTCTCCCACCTGTTCTCCTGAAA
AGCCCGGTCCCTCTTCCTCTGCCTCTTCCATGGGCCCAGACCTGAGCAGAACCG
GGCTTACCCAGCTGCCCAGAGAGACTCTAGGGGCCAGAAGCCATGCCCCAGAG
CTCCCAGGC**G/A**GGGCTGCCACCCGGGAACTTCTGCTTCAAGCTTCAGGGTAG
ACACAGAGACCTGGATGAGACTCAAGTCCCCTCCCTGATCCTGGGCCTGCTGAA
GCCCCTTCCTCCATGGCTCCTGTCATGAGAGCAAAACATTGTCGCCTGGAGACG
GTGACTCGGAAAGCCTCCTGGGAGACAGGAAAGGCATCGATGCCACAGCTCCA
TCCTCTACTTGACCCTTGCTGGCGGGAGGGGAGTGATATGTCCACACACTGCAC
TGCGTCATCCTGTTCCGGATGCCTCCGGAGAGAGGGACGGACAGTCAGAAACA
AGGGAGTTTCTATTAAAGGCCATCCAAACCAAAAAAAAAAAAAAAAAAA

Figure 3 4/10

Wild Type Equine LH1 peptide (SEQ ID NO:3)

MRPLLLLAPLGWLLLAEAKGDAKPEDNLLVLTVATKESEGFRRFKRSAQFFNYKIQA
LGLGEDWDGDKETSAGGGLKVRLLKKALEKHADKENLVILFTDSYDVVFASGPRELLKKF
RQARSQVVFSAEELIYPDRRLEAKYPVVSDGKRFLGSGGFIGYAPNLSKLVAEWEGQDSD
SDQLFYTKIFLDPEKRERINITLDHRCRIFQNLVGALDEVVLKFEMGHVRARNLAYDTLP
VLIHGNGPTKLQLNYLGNYIPRFWTFETGCTVCDEGLRSLKGIGDEALPVVLVGVFIEQP
TPFLSLFFQRLLRLHYPRKQLRLFIHNHEQHHKAQVEQFLAEHGGEYKSVKLVGPEVRVA
NADARNMGADLCRQDRGCTYYFSVDADVALTEPKTLRLLIEQNKNVIAPLMTRHGRLWSN
FWGAMSADGYYARSEDYVDIVQGRRVGVWNVPYISNIYLIKGSALRAELQQTDLFHHSKL
DADMAFCANIRQQDVFMFLTNRHTFGHLLSLDSYQTTHLHNDLWEVFSNPEDWKEKYIHE
NYTRALAGKLVEMPCPDVYWFPIFTETACDELVEEMEHYGQWSLGDNKDNRIQGGYENVP
TIDIHMNQISFEREWHKFLVEYIAPMTEKLYPGYYTRAQFDLAFVVRYKPDEQPSLMPHH
DASTFTVNIALNRVGVDYEG**G**GCRFLRYNCSIRAPRKGWTLMHPGRLTHYHEGLPTTKGTRYIAVSFVDP

Figure 4 5/10

Mutant Equine LH1 cDNA. (SEQ ID NO:4)

AGTTTCCAGCCTGCCAGCGCCTGCTGGCTGCCCGGACGTCCCCAGACCTCGGCC**A**TGCGGCCTCT
GCTGCTCCTGGCCCCGCTGGGCTGGCTGCTCCTGGCCGAAGCGAAGGGCGACGCCAAGCCGGAG
GACAACCTCTTAGTCCTCACGGTGGCCACGAAGGAGTCCGAGGGGTTCCGACGCTTCAAGCGCT
CAGCCCAGTTCTTCAACTACAAGATCCAGGCGCTGGGCCTGGGGGAGGACTGGGACGGGGACAA
GGAGACGTCAGCGGGCGGCGGGCTGAAGGTTCGGCTGCTGAAGAAAGCTCTGGAGAAGCATGC
AGACAAAGAGAACCTGGTCATTCTCTTCACAGACAGCTATGATGTGGTATTTGCCTCGGGGCCCC
GAGAGCTCCTGAAGAAGTTCCGGCAGGCCAGGAGCCAGGTGGTCTTCTCGGCCGAGGAGCTCAT
CTACCCCGACCGCAGGCTGGAGGCCAAGTACCCGGTGGTGTCCGATGGCAAGAGGTTCCTGGGC
TCTGGAGGCTTCATCGGTTATGCCCCCAACCTCAGCAAACTGGTGGCTGAGTGGGAGGGCCAGG
ACAGCGACAGTGACCAGCTGTTTTATACCAAGATCTTCTTGGACCCAGAGAAGAGGGAGCGGAT
CAACATCACCCTGGACCACCGCTGCCGTATCTTCCAGAACCTGGTTGGAGCCTTAGATGAGGTCG
TGCTCAAGTTTGAAATGGGCCATGTGAGGGCGCGGAACCTGGCCTACGACACCCTCCCCGTCCTG
ATTCATGGCAACGGGCCCACCAAGCTGCAGCTGAACTACCTGGGCAACTATATCCCTCGCTTCTG
GACCTTCGAGACGGGCTGCACGGTGTGTGACGAGGGCCTGCGCAGCCTCAAGGGCATTGGGGAT
GAAGCTCTGCCTGTGGTCTTGGTCGGCGTGTTCATCGAGCAGCCCACGCCGTTCCTGTCCCTGTTC
TTCCAGCGGCTTCTGCGCCTGCATTACCCCCGGAAACAGCTGCGGCTTTTTATTCATAACCATGA
GCAGCACCACAAGGCTCAGGTGGAGCAGTTCCTGGCAGAGCATGGCGGCGAGTACAAGTCTGTG
AAACTGGTGGGCCCCGAGGTGCGGGTGGCAAACGCCGATGCCAGGAACATGGGCGCGGACCTGT
GCCGGCAGGACCGTGGCTGCACCTACTACTTCAGTGTGGATGCCGACGTGGCCCTGACCGAGCC
CAAGACCCTGCGACTGCTGATTGAGCAGAACAAGAATGTCATCGCCCCGTTGATGACCCGCCAC
GGGAGGCTGTGGTCGAACTTCTGGGGGGCAATGAGTGCAGATGGCTACTACGCCCGCTCCGAGG
ACTACGTGGACATTGTGCAGGGGCGGCGTGTTGGCGTCTGGAACGTGCCCTACATCTCGAACATT
TACCTGATCAAGGGCAGTGCCCTGCGGGCTGAGCTGCAGCAGACAGATCTGTTCCACCACAGCA
AGCTGGATGCCGACATGGCCTTCTGTGCCAATATCCGGCAGCAGGATGTGTTCATGTTCCTGACC
AACCGGCACACCTTCGGCCACCTGCTCTCCCTGGACAGCTACCAGACCACCCACCTCCACAACGA
CCTCTGGGAGGTGTTTAGCAACCCCGAGGACTGGAAGGAGAAGTACATCCATGAGAACTACACC
AGGGCCCTGGCGGGGAAGCTGGTGGAGATGCCTTGCCCGGATGTCTACTGGTTCCCCATCTTCAC
GGAGACGGCCTGTGACGAGCTGGTGGAGGAGATGGAGCACTACGGCCAGTGGTCTCTGGGAGAC
AATAAGGACAACCGCATCCAGGGTGGCTACGAAAATGTGCCGACCATCGACATCCACATGAACC
AGATCAGCTTTGAGCGGGAGTGGCACAAGTTCCTGGTGGAGTACATCGCCCCCATGACAGAGAA
GCTGTATCCAGGCTACTACACCAGGGCCCAGTTCGACCTGGCCTTTGTTGTCCGCTACAAGCCTG
ACGAGCAGCCCTCGCTGATGCCCCACCACGATGCCTCCACCTTCACTGTCAACATCGCCCTGAAC

CGGGTCGGGGTGGATTACGAGGGC**A**GGGGCTGTCGGTTCCTGCGTTACAACTGCTCCATCCGAG
CCCCACGGAAGGGCTGGACCCTCATGCACCCCGGACGACTCACGCACTACCACGAGGGACTCCC
CACCACCAAGGGCACCCGCTACATCGCGGTCTCCTTCGTCGATCCC**TAA**TCGGCCAAGCCTGGC
CACTTCGGACCTTTTCCTCGTTGCCAACAACCACTGCCCAGCAGCCTCTGGGGCCTTGGGGTCCC
AGGGAACCTGGTCCAGCCTCCGGGCTCTTGACCTCCCATTGCTTTCGGAGCCGCC**A**TCGGAGAGA
CTGGGCCGCAGGCCAGAGGCAGAGCACACCTCCTTGGCTGGGGCTTTCCTGGTGTTCTGCTCCCC
ACCCCGGGAGATGGGGTCCACGCTCACTGCCTTGTAACAGCTCATCCTCTCCCACCTGTTCTCCT
GAAAAGCCCGGTCCCTCTTCCTCTGCCTCTTCCATGGGCCCAGACCTGAGCAGAACCGGGCTTAC
CCAGCTGCCCAGAGAGACTCTAGGGGCCAGAAGCCATGCCCCAGAGCTCCCAGGC**A**GGGCTGCC
ACCCGGGAACTTCTGCTTCAAGCTTCAGGGTAGACACAGAGACCTGGATGAGACTCAAGTCCCC
TCCCTGATCCTGGGCCTGCTGAAGCCCCTTCCTCCATGGCTCCTGTCATGAGAGCAAAACATTGT
CGCCTGGAGACGGTGACTCGGAAAGCCTCCTGGGAGACAGGAAAGGCATCGATGCCACAGCTCC
ATCCTCTACTTGACCCTTGCTGGCGGGAGGGGAGTGATATGTCCACACACTGCACTGCGTCATCC
TGTTCCGGATGCCTCCGGAGAGAGGGACGGACAGTCAGAAACAAGGGAGTTTCTATTAAAGGCC
ATCCAAACCAAAAAAAAAAAAAAAAAAA

Figure 5 6/10

Mutant Equine LH1 peptide (SEQ ID NO:5)

MRPLLLLAPLGWLLLAEAKGDAKPEDNLLVLTVATKESEGFRRFKRSAQFFNYKIQA
LGLGEDWDGDKETSAGGGLKVRLLKKALEKHADKENLVILFTDSYDVVFASGPRELLKKF
RQARSQVVFSAEELIYPDRRLEAKYPVVSDGKRFLGSGGFIGYAPNLSKLVAEWEGQDSD
SDQLFYTKIFLDPEKRERINITLDHRCRIFQNLVGALDEVVLKFEMGHVRARNLAYDTLP
VLIHGNGPTKLQLNYLGNYIPRFWTFETGCTVCDEGLRSLKGIGDEALPVVLVGVFIEQP
TPFLSLFFQRLLRLHYPRKQLRLFIHNHEQHHKAQVEQFLAEHGGEYKSVKLVGPEVRVA
NADARNMGADLCRQDRGCTYYFSVDADVALTEPKTLRLLIEQNKNVIAPLMTRHGRLWSN
FWGAMSADGYYARSEDYVDIVQGRRVGVWNVPYISNIYLIKGSALRAELQQTDLFHHSKL
DADMAFCANIRQQDVFMFLTNRHTFGHLLSLDSYQTTHLHNDLWEVFSNPEDWKEKYIHE
NYTRALAGKLVEMPCPDVYWFPIFTETACDELVEEMEHYGQWSLGDNKDNRIQGGYENVP
TIDIHMNQISFEREWHKFLVEYIAPMTEKLYPGYYTRAQFDLAFVVRYKPDEQPSLMPHH
DASTFTVNIALNRVGVDYEG**R**GCRFLRYNCSIRAPRKGWTLMHPGRLTHYHEGLPTTKGTRYIAVSF
VDP

Figure 6

Wild Type Equine LH1 Exon 19 (SEQ ID NO:6)

GGC**G**GGGGCTGTCGGTTCCTGCGTTACAACTGCTCCATCCGAGCCCCACGGAA
GGGCTGGACCCTCATGCACCCCGGACGACTCACGCACTACCACGAGGGACTCC
CCACCACCAAGGGCACCCGCTACATCGCGGTCTCCTTCGTCGATCCCTAATCGG
CCAAGCCTGGCCACTTCGGACCTTTTCCTCGTTGCCAACAACCACTGCCCAGCA
GCCTCTGGGGCCTTGGGGTCCCAGGGAACCTGGTCCAGCCTCCGGGCTCTTGAC
CTCCCATTGCTTTCGGAGCCGCC**G/A**TCGGAGAGACTGGGCCGCAGGCCAGAG
GCAGAGCACACCTCCTTGGCTGGGGCTTTCCTGGTGTTCTGCTCCCCACCCCGG
GAGATGGGGTCCACGCTCACTGCCTTGTAACAGCTCATCCTCTCCCACCTGTTCT
CCTGAAAAGCCCGGTCCCTCTTCCTCTGCCTCTTCCATGGGCCCAGACCTGAGC
AGAACCGGGCTTACCCAGCTGCCCAGAGAGACTCTAGGGGCCAGAAGCCATGC
CCCAGAGCTCCCAGGC**G/A**GGGCTGCCACCCGGGAACTTCTGCTTCAAGCTTCA
GGGTAGACACAGAGACCTGGATGAGACTCAAGTCCCCTCCCTGATCCTGGGCCT
GCTGAAGCCCCTTCCTCCATGGCTCCTGTCATGAGAGCAAAACATTGTCGCCTG
GAGACGGTGACTCGGAAAGCCTCCTGGGAGACAGGAAAGGCATCGATGCCACA
GCTCCATCCTCTACTTGACCCTTGCTGGCGGGAGGGGAGTGATATGTCCACACA
CTGCACTGCGTCATCCTGTTCCGGATGCCTCCGGAGAGAGGGACGGACAGTCAG
AAACAAGGGAGTTTCTATTAAAGGCCATCCAAACC

Figure 7 7/10

Mutant Equine LH1 Exon 19 (SEQ ID NO:7)

GGC**A**GGGGCTGTCGGTTCCTGCGTTACAACTGCTCCATCCGAGCCCCACGGAA
GGGCTGGACCCTCATGCACCCCGGACGACTCACGCACTACCACGAGGGACTCC
CCACCACCAAGGGCACCCGCTACATCGCGGTCTCCTTCGTCGATCCCTAATCGG
CCAAGCCTGGCCACTTCGGACCTTTTCCTCGTTGCCAACAACCACTGCCCAGCA
GCCTCTGGGGCCTTGGGGTCCCAGGGAACCTGGTCCAGCCTCCGGGCTCTTGAC
CTCCCATTGCTTTCGGAGCCGCC**A**TCGGAGAGACTGGGCCGCAGGCCAGAGGC
AGAGCACACCTCCTTGGCTGGGGCTTTCCTGGTGTTCTGCTCCCCACCCCGGGA
GATGGGGTCCACGCTCACTGCCTTGTAACAGCTCATCCTCTCCCACCTGTTCTCC
TGAAAAGCCCGGTCCCTCTTCCTCTGCCTCTTCCATGGGCCCAGACCTGAGCAG
AACCGGGCTTACCCAGCTGCCCAGAGAGACTCTAGGGGCCAGAAGCCATGCCC
CAGAGCTCCCAGGC**A**GGGCTGCCACCCGGGAACTTCTGCTTCAAGCTTCAGGG
TAGACACAGAGACCTGGATGAGACTCAAGTCCCCTCCCTGATCCTGGGCCTGCT
GAAGCCCCTTCCTCCATGGCTCCTGTCATGAGAGCAAAACATTGTCGCCTGGAG
ACGGTGACTCGGAAAGCCTCCTGGGAGACAGGAAAGGCATCGATGCCACAGCT
CCATCCTCTACTTGACCCTTGCTGGCGGGAGGGGAGTGATATGTCCACACACTG
CACTGCGTCATCCTGTTCCGGATGCCTCCGGAGAGAGGGACGGACAGTCAGAA
ACAAGGGAGTTTCTATTAAAGGCCATCCAAACC

Figure 8 8/10

Equine LH1 Exon 1: (SEQ ID NO:8)

AGTTTCCAGCCTGCCAGCGCCTGCTGGCTGCCCGGACGTCCCCAGACCTCGGCC
ATGCGGCCTCTGCTGCTCCTGGCCCCGCTGGGCTGGCTGCTCCTGGCCGAAGCG
AAGGGCGACGCCAAGCCGGAGG

Equine LH1 Exon 2: (SEQ ID NO:9)

ACAACCTCTTAGTCCTCACGGTGGCCACGAAGGAGTCCGAGGGGTTCCGACGCT
TCAAGCGCTCAGCCCAGTTCTTCAACTACAAGATCCA

Equine LH1 Exon 3: (SEQ ID NO:10)

GGCGCTGGGCCTGGGGGAGGACTGGGACGGGGACAAGGAGACGTCAGCGGGC
GGCGGGCTGAAGGTTCGGCTGCTGAAGAAAGCTCTGGAGAAGCATGCAGACAA
AGAGAACCTGGTCATTCTCTTCACAGACAG

Equine LH1 Exon 4: (SEQ ID NO:11)

CTATGATGTGGTATTTGCCTCGGGGCCCCGAGAGCTCCTGAAGAAGTTCCGGCA
GGCCAGGAGCCAGGTGGTCTTCTCGGCCGAGGAGCTCATCTACCCCGACCGCA
GGCTGGAGGCCAAGTACCCGGTGGTGTCCGATGGCAAGAGGTTCCTGGGCTCT
GGAG

Equine LH1 Exon 5: (SEQ ID NO:12)

GCTTCATCGGTTATGCCCCCAACCTCAGCAAACTGGTGGCTGAGTGGGAGGGCC
AGGACAGCGACAGTGACCAGCTGTTTTATACCAAGATCTTCTTGGACCCAGAGA
AGAGG

Equine LH1 Exon 6: (SEQ ID NO:13)

GAGCGGATCAACATCACCCTGGACCACCGCTGCCGTATCTTCCAGAACCTGG
TTGGAGCCTTAG

Equine LH1 Exon 7: (SEQ ID NO:14)

ATGAGGTCGTGCTCAAGTTTGAAATGGGCCATGTGAGGGCGCGGAACCTGGCC
TACGACACCCTCCCCGTCCTGATTCATGGCAACGGGCCCACCAAG

Equine LH1 Exon 8: (SEQ ID NO:15)

CTGCAGCTGAACTACCTGGGCAACTATATCCCTCGCTTCTGGACCTTCGAGACG
GGCTGCACGGTGTGTGACGAGGGCCTGCGCAGCCTCAAGGGCATTGGG

Figure 8, continued

Equine LH1 Exon 9: (SEQ ID NO:16)

GATGAAGCTCTGCCTGTGGTCTTGGTCGGCGTGTTCATCGAGCAGCCCACGCCG
TTCCTGTCCCTGTTCTTCCAGCGGCTTCTGCGCCTGCATTACCCCCGGAAACAGC
TGCGGCTTTTTATTCATAACCAT

Equine LH1 Exon 10: (SEQ ID NO:17)

GAGCAGCACCACAAGGCTCAGGTGGAGCAGTTCCTGGCAGAGCATGGCGGCGA
GTACAAGTCTGTGAAACTGGTGGGCCCCGAGGTGCGGGTGGCAAACGCCGATG
CCAGGAACATGGGCGC

Equine LH1 Exon 11: (SEQ ID NO:18)

GGACCTGTGCCGGCAGGACCGTGGCTGCACCTACTACTTCAGTGTGGATGCCGA
CGTGGCCCTGACCGAGCCCAAGACCCTGCGACTGCTGATTGAGCAGAACAA

Equine LH1 Exon 12: (SEQ ID NO:19)

GAATGTCATCGCCCCGTTGATGACCCGCCACGGGAGGCTGTGGTCGAACTTCTG
GGGGGCAATGAGTGCAGATGGCTACTACGCCCGCTCCGAGGACTACGTGGACA
TTGTGCAGGGGCGGCGTGT

Equine LH1 Exon 13: (SEQ ID NO:20)

TGGCGTCTGGAACGTGCCCTACATCTCGAACATTTACCTGATCAAGGGCAGTGC
CCTGCGGGCTGAGCTGCAGCAGACAGATCTGTTCCACCACAGCAAGCTGGATG
CCGACATGGCCTTCTGTGCCAATATCCGGCAGCAG

Equine LH1 Exon 14: (SEQ ID NO:21)

GATGTGTTCATGTTCCTGACCAACCGGCACACCTTCGGCCACCTGCTCTCCCTGG
ACAGCTACCAGACCACCCACCTCCACAACGACCTCTGGGAGGTGTTTAGCAACC
CCGAGGAC

Equine LH1 Exon 15: (SEQ ID NO:22)

TGGAAGGAGAAGTACATCCATGAGAACTACACCAGGGCCCTGGCGGGGAAGCT
GGTGGAGATG

Figure 8, continued 10/10

Equine LH1 Exon 16: (SEQ ID NO:23)

CCTTGCCCGGATGTCTACTGGTTCCCCATCTTCACGGAGACGGCCTGTGACGAG
CTGGTGGAGGAGATGGAGCACTACGGCCAGTGGTCTCTGGGAGACAATAAG

Equine LH1 Exon 17: (SEQ ID NO:24)

GACAACCGCATCCAGGGTGGCTACGAAAATGTGCCGACCATCGACATCCACAT
GAACCAGATCAGCTTTGAGCGGGAGTGGCACAAGTTCCTGGTGGAGTACATCG
CCCCCATGACAGAGAAGCTGTATCCAGGCTACTACACCAGG

Equine LH1 Exon 18: (SEQ ID NO:25)

GCCCAGTTCGACCTGGCCTTTGTTGTCCGCTACAAGCCTGACGAGCAGCCCTCG
CTGATGCCCCACCACGATGCCTCCACCTTCACTGTCAACATCGCCCTGAACCGG
GTCGGGGTGGATTACGAG

Figure 9A (SEQ ID NO:26)

atagctgt cactccacaa ggcacaaggg ctgcttggct ggggtggctc
agatgggaga atgggggta ctaggggaag ggcccagctt cctcttgggg aaactgacgc
ttcctgttgg gaaactgaca cttcctgtct cccagggc**g**g gggctgtcgg ttcctgcgtt
acaactgctc catccgagcc ccacggaagg gctggaccct catgcacccc ggacgactca
cgcactacca cgagggactc cccaccacca agggcacccg ctacatcgcg gtctccttcg
tcgatcccta atcggccaag cctggccact tcggaccttt tcctcgttgc caacaaccac
tg

Figure 9B (SEQ ID NO:1)

atagctgt cactccacaa ggcacaaggg ctgcttggct ggggtggctc
agatgggaga atgggggta ctaggggaag ggcccagctt cctcttgggg aaactgacgc
ttcctgttgg gaaactgaca cttcctgtct cccagggc**a**g gggctgtcgg ttcctgcgtt
acaactgctc catccgagcc ccacggaagg gctggaccct catgcacccc ggacgactca
cgcactacca cgagggactc cccaccacca agggcacccg ctacatcgcg gtctccttcg
tcgatcccta atcggccaag cctggccact tcggaccttt tcctcgttgc caacaaccac
tg

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/37981

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68 (2012.01)
USPC - 435/6.12
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC: 435/6.12
IPC(8): C12Q 1/68 (2012.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 435/6.1, 435/23.1

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (DB=PGPB,USPT,USOC,EPAB,JPAB; PLUR=NO; OP=ADJ), Google Scholar, Google Patents
Search Terms Used: fragile foal syndrome, wffstl, lysyl hydroxylase horse, equine, Winand, Cornell

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2009/0258360 A1 (VALBERG et al.) 15 October 2009 (15.10.2009) esp: paras [0011], [0062], [0126], [0169], [0185], [0189].	1-14
A	GenBank Deposition CX595913 - CT020012B10E10 Equine Articular Cartilage cDNA Library Equus caballus cDNA clone CT020012B10E10, mRNA sequence, 30 December 2010 (30.12.2010) [online]. [Retrieved on 6 August 2012]. Retrieved from the internet <URL: http://www.ncbi.nlm.nih.gov/nucest/CX595913> entire document	1-5, 15
A	GenBank Deposition CX597645 - CT020018B10E01 Equine Articular Cartilage cDNA Library Equus caballus cDNA clone CT020018B10E01, mRNA sequence, 30 December 2010 (30.12.2010) [online]. [Retrieved on 6 August 2012]. Retrieved from the internet <URL: http://www.ncbi.nlm.nih.gov/nucest/CX597645>entire document	6-14
A, P	US 2011/0265193 A1 (BROOKS et al.) 27 October 2011 (27.10.2011) entire document.	1-14
A	US 2004/0137470 A1 (DHALLAN) 15 July 2004 (15.07.2004) entire document.	1-14
A	WO 2008/121727 A1 (WINAND) 9 October 2008 (09.10.2008) entire document.	1-14

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"A" document defining the general state of the art which is not considered to be of particular relevance
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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
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Date of the actual completion of the international search	Date of mailing of the international search report
6 August 2012 (06.08.2012)	31 AUG 2012

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